Volume XXXI January, 1931 Number 1

SOIL SCIENCE

Contents

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
BALTIMORE, MARYLAND, U. S. A.

Made in United States of America

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

SOIL SCIENCE

VOLUME XXXI

JANUARY – JUNE, 1931

RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY
U. S. A.

PUBLISHED BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MARYLAND

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879

CONTENTS

ILLUSTRATIONS

PLATES

CONSERVATION AND AVAILABILITY OF THE NITROGEN IN FARM MANURE

A METHOD OF APPLICATION DESIGNED TO INSURE PROPER DISTRIBUTION OF FERTILIZERS IN FIELD TRIALS WITH FRUIT TREES

THE AVAILABILITY OF THE NITROGEN IN FARM MANURE UNDER FIELD CONDITIONS

THE MODELING OF SOILS, AN AID IN THEIR STUDY

TEXT-FIGURES

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: IV. ISOELECTRIC PRECIPITATES

THE TEMPERATURE CORRECTION IN THE HYDROMETER METHOD OF MECHANICAL ANALYSIS OF SOILS

SOIL SAMPLING WITH A COMPRESSED AIR UNIT

H. F. BLANEY AND C. A. TAYLOR

U. S. Department of Agriculture

Received for publication April 14, 1930

In connection with investigations on rainfall penetration in southern California, the authors[1] are conducting soil moisture studies in which soil samples are taken, at 1-foot intervals to a depth of 18 feet, with the improved soil tube.[2] The success of this work depends, to a considerable extent, upon the speed with which a large number of samples can be obtained, and since the physical effort required may become the limiting factor in obtaining samples at considerable depths, a compressed air unit has been adapted for use in driving the tubes in order that as many samples as desired can be taken quickly and easily.

The apparatus consists of a compressor unit mounted on a truck, a light air hammer, and a 100-foot length of hose. Plate 1 shows the mounting of the apparatus and the manner in which soil tubes are driven. The compressor unit consists of a 2-cylinder air compressor coupled directly to a 4-cylinder gasoline engine with a self-starter, the air receiver and gasoline tank being mounted in front of the compressor. The complete unit is mounted on a channel iron frame and bolted to one side of the truck floor, thus leaving one-half of the truck body space available for carrying other equipment. A reel with 100 feet of ½-inch rubber hose is mounted at the back of the truck and special hose couplings permit connections to be quickly made to the hammer and compressor. An air pressure control automatically maintains any desired pressure up to 150 pounds to the square inch and the displacement of the compressor is 59 cubic feet a minute at 800 revolutions a minute.

The hammer is of the clay digger type, capable, when working under an air pressure of 100 pounds to the square inch, of delivering 2,250 blows a minute, each blow striking with a force of 16 foot-pounds. Extending down into the soil tube is a 6-inch guide rod, the shoulder of which may be seen resting against the end of the soil tube in figure 2 of plate 1. The trigger grip gives the operator ready control of the hammer, and by properly cramping the guide rod in the end of the soil tube, allows very little vibration to be transmitted to the arms of the operator. The jack[3] used for pulling the soil tubes is shown on the ground in figure 1 of plate 1.

[1] Under the supervision of W. W. McLaughlin, Associate chief of the division of agricultural engineering, Bureau of Public Roads, U. S. Department of Agriculture, in coöperation with the State of California, Department of Public Works.

[2] Veihmeyer, F. J. 1929 An improved soil-sampling tube, *Soil Sci.* 27: 147–152.

[3] Taylor, C. A., and Blaney, H. F., 1929. An efficient soil tube jack. *Soil Sci.* 27: 351–353.

The set-up for sampling is quickly made, as it is merely necessary to reel the hose out to the desired location and snap it on the compressor. This unit has been in use since October, 1929, and has proved very satisfactory. It is estimated that the time of sampling has been cut to one-third of that required for hand work.

PLATE 1

COMPRESSED AIR UNIT FOR SOIL SAMPLING

FIG. 1. Compressor unit and air hammer in operation.
FIG. 2. Detail view of air hammer.

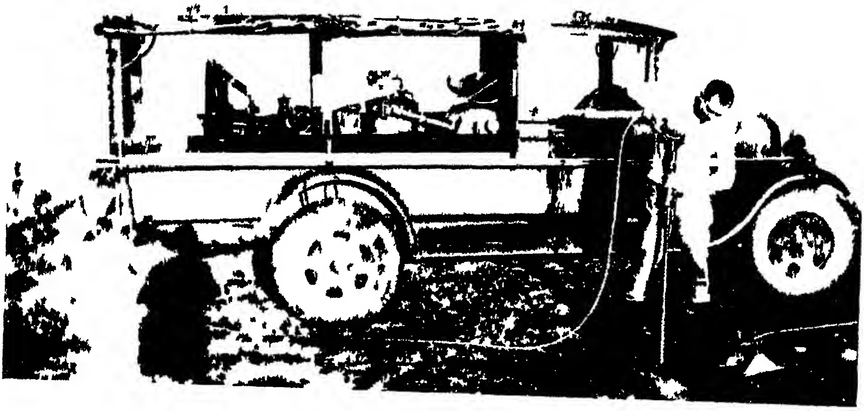


FIG. 1

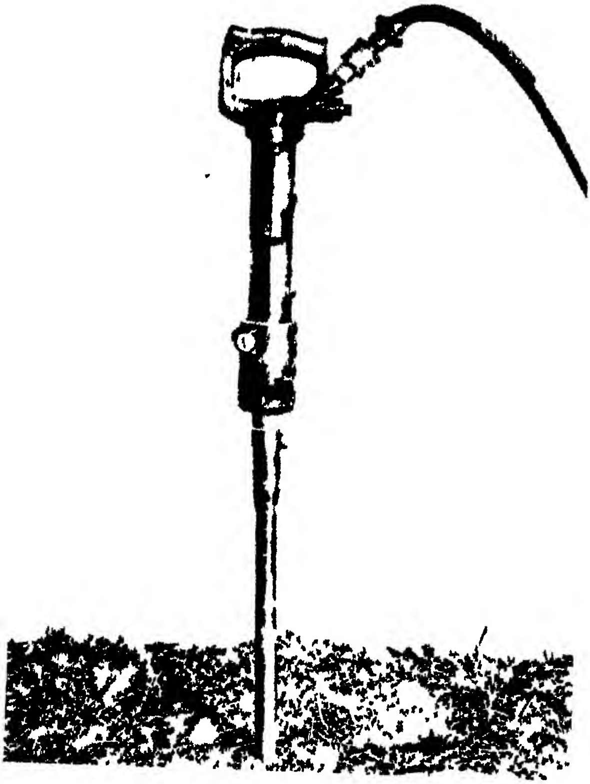


FIG. 2

AMMONIUM CALCIUM BALANCE: A CONCENTRATED FERTILIZER PROBLEM[1]

L. G. WILLIS AND J. R. PILAND[2]

North Carolina Agricultural Experiment Station

Received for publication April 17, 1930

Although much soil fertility research has dealt with the availability and efficiency of the separate ingredients of fertilizers as well as of mixtures, the problem of actual injury to plants resulting from the use of fertilizers has also demanded attention.

In the early development of the fertilizer industry the use of light applications of low grade, low analysis materials required that the major research effort be exerted on the former of these two problems, whereas the recent trend toward more liberal fertilization, and the perfection of chemical processes for the production of fertilizer ingredients of greater solubility and availability has made the latter problem more acute. This is particularly true as it concerns sandy soil types of low absorptive capacity.

Undoubtedly, plasmolysis of plant root tissues is responsible for some of the occurrences of injury consequent to the use of soluble salts as fertilizers, but numerous observations point to other principles governing fertilizer toxicity. As Beaumont (2) points out, the soluble fertilizer materials more highly concentrated in respect to plant nutrients would be less likely to cause injury to plants through plasmolysis than would the cruder salts or those of lower analysis furnishing the same quantity of nutrients. But the same writer (1) reports a reduction in stand of sweet corn and potatoes following the use of high analysis fertilizers in comparison with ordinary materials furnishing equal quantities of nitrogen, phosphorus, and potassium. It was observed, in this case, that the injury, which was limited to the young plants, might be avoided by thoroughly mixing the fertilizer with the soil. The work of Skinner, Williams and Mann (14) has shown a similar injury to cotton seedlings following the use of concentrated fertilizers on Coastal Plain sandy soils, and Skinner (13) has summarized some other observations on this effect of high analysis materials.

Increasing the concentration of nitrogen, phosphorus, and potassium in fertilizers tends to eliminate other elements, and the practicable possibilities in this direction may not be exceeded by mixtures where potassium and ammo-

[1] Publication as no. 41 of the Journal Series authorized by the director of the agricultural experiment station.

[2] Soil chemist and assistant, respectively.

nium are the only cations present. Under such conditions there is the possibility that not only plasmolysis but also the physiological toxicity of these ions may be a factor in fertilizer injury. This type of toxicity, although extensively studied in solution cultures and in relation to alkali soil problems has apparently received little consideration as regards its applicability to fertilization practices.

The physiological toxicity of solutions of single salts is evidenced by abnormal changes in the permeability of the plant cells, or in extreme cases, by disintegration of the tissues. This condition of increased permeability as well as the toxic effects of the solutions of single salts is averted or antagonized by certain salt combinations. In general it has been found that the greatest degree of antagonism exists between salts with monovalent and those with bivalent cations, especially calcium. According to Osterhout (8) this antagonistic effect is due to the presence in the cell of a compound regulating permeability. Toxic and antagonistic salts affect permeability as they accelerate or retard the formation or decomposition of this compound.

Raber (9) explains the phenomenon on the basis of the effect of the electrical charge of the salt on the protoplasm of the cell. The protoplasm being negatively charged, highly positive salts, such as those with bivalent cations, coagulate the protoplasm and thereby decrease permeability, whereas the less positive salts, as typified by those with monovalent cations, increase permeability. Raber's hypothesis, by taking into consideration the electrical properties of the anion as well as the cation, provides an explanation for the variation in the effects of different salts with a common base. The balancing of these electrical effects so as to produce no abnomality in the permeability of the cell is considered the basis of antagonism.

Another theory, that of Hansteen-Cranner (5), is based on evidence of the effect of solutions on the lipoid and pectic materials which govern the peameability of the cell walls. According to this theory the toxic action of the alkalis is due to the dispersion of these components of the cell wall whereas the antagonistic effect of calcum ions results from the coagulative effect of that element.

On strictly thoretical grounds, therefore, the use of fertilizers containing only potassum and ammonium as cations involves the risk of injury to plants. In many cases the rapid absorption of these bases by the soil or an abundant supply of soil calcium would insure safety in the use of such fertilizers, but for soils lacking these features the inclusion of a salt of calcium in the formula might result in a more satisfactory plant response.

Another possible cause of injury to seedling plants may lie in the use of nitrogenous materials easily decomposable in the soil into free ammonia.

Sand cultures containing 10 p.p.m. of nitrogen as free ammonia were found by Russell and Petherbridge (11) to regard the germination of turnip seed whereas with 100 p.p.m. of nitrogen no seed germinated. Since the sand contained 16.7 per cent of moisture it appears that 0.006 per cent of nitrogen as free ammonia is injurious and that 0.06 per cent is fatal.

It is certain, however, that free ammonia would be promptly converted to the

carbonate in any soil; therefore all evidence of the toxicity of free ammonia is in support of the observation of Darwin (3), as quoted by Ehrenberg (4), that ammonium carbonate is injurious to plants.

The possibility of the application of these facts to some concentrated fertilizer problems has been studied by attempting to produce an injury from the use of various materials and to test methods of control based on the foregoing hypotheses.

EXPERIMENTAL

Of the many possible formulas representative of concentrated fertilizers, a mixture of diammonium phosphate, potassium nitrate, and potassium chloride in amounts supplying nitrogen, phosphoric acid, and potash in a 1:2:1 ratio was arbitrarily chosen as representing a practical commercial formula. Ordinary C. P. chemicals were used, being reground when necessary to permit uniform distribution.

A very incoherent Norfolk sand which is being successfully farmed was taken for pot culture experiments and cotton seedlings were used as test plants. The following method of experimentation was used throughout the work:

The soil was wetted to a degree that would insure the germination of the seed without being too wet to be handled. Six and a half pounds of soil was placed into each of enough 1-gallon glazed earthenware pots to provide duplications of the projected treatments. The soil was moderately compacted and the surface levelled.

On this surface, the dry and finely powdered freshly mixed fertilizer materials were distributed as uniformly as possible. Two and a half pounds of soil was added and compacted. Fifteen seeds to each pot were planted with uniform spacing. On this, one pound of soil was spread loosely, and the pots were set to within an inch of their tops in sawdust on a greenhouse bench and covered with waterproof paper. When the seedlings appeared, the paper was removed and distilled water, if necessary, was added uniformly.

As soon as the primary leaves had opened fully, usually in eight or nine days, the soil was washed out of the pots through a screen and the condition of the plants noted. Injury was recorded in those cases where the tap roots of the seedlings had rotted off at the plane of fertilizer application. There was no evidence that germination was in any way affected by the treatments and the percentage of injury is computed from the number of plants having roots extending to or through the fertilizer application.

Toxicity of fertilizer ingredients

The toxicity of the complete fertilizer and of each ingredient and the antagonistic effects of supplementary treatments were tested in duplicate pot experiments.

One and one-half grams to each pot of a mixture containing 0.98 gm. of diammonium phosphate, 0.40 gm. of potassium nitrate, and 0.12 gm. of potassium chloride was used as the basal complete fertilizer. This was approximately equal to a field rate of application of 96 pounds an acre in the drill, of a fertilizer analyzing 17.5 per cent of nitrogen, 35.0 per cent of phosphoric acid, and 17.5 per cent of potash.

At this rate 0.02 of the gram-atomic weight of the combined cations was

supplied to each pot, and each ingredient was tested separately as to toxicity and response to supplementary treatments at the same equivalent concentration as that of the whole formula. Thus, the separate ingredients were used in the following amounts for each pot: diammonium phosphate 1.34 gm., potassium nitrate 2.05 gm., and potassium chloride 1.51 gm.

Supplementary treatments were added in amounts equivalent to one-fifth of the cation concentration of the fertilizer mixture as follows:

	gm.		gm.
Na_2SO_4	.28	$CaSO_4 \cdot 2H_2O$	.69
K_2SO_4	.35	$MgSO_4 \cdot 7H_2O$	.99
$NaCl$	.23	$CaCl_2 \cdot 2H_2O$	.59
KCl	.30	$MgCl_2 \cdot 6H_2O$	.81

The rate of application of the supplementary treatments was chosen on the basis of observations made in preliminary work on the problem. It does not represent any optimum ratio but only an amount that had been found to establish the effectiveness of the material in controlling toxicity. Differences in solubility and in degrees of ionization of the materials in solution as well as of absorption by the soil would modify considerably the relative concentration of ions to which the seedling plants were exposed. Such differences appear unavoidable and probably do not invalidate the results.

The plan of the experiment and the results are given in table 1.

The data of table 1 demonstrate very strikingly the fact that the complete mixture is toxic to cotton seedlings and that the toxicity is reduced by the addition of calcium chloride but not by sodium or potassium chloride. Apparently magnesium chloride is not quite so efficient in this respect as is the calcium salt but on account of the high degree of variability of the results from duplicate pots minor differences in percentages of injury are of questionable significance. The type of injury and degree of control are illustrated in plate 1, showing the seedlings from duplicate pots on either side of the reference number. None of the supplementary treatments was injurious at the rates of application used.

With the diammonium phosphate, injury was evidenced by the failure of any roots to penetrate below the plane of fertilization. The remedial effect of magnesium salts was almost negligible and that of the calcium salts was distinctly less than when used with the complete mixture. A reasonable explanation of this difference may be found in the fact that the concentration of diammonium phosphate in the mixed fertilizer was less than that of the same component used alone, whereas the concentration of the supplementary treatment was the same for both.

Potassium nitrate was moderately injurious alone and with all supplementary treatments; there was no conclusive evidence of an antagonism similar to that found with the diammonium phosphate. Potassium chloride was non-toxic alone and in all mixtures.

The effectiveness of the calcium and magnesium salts in correcting such toxicity might be acceptable as evidence of simple antagonism of the ammon-

TABLE 1

Injurious effect of a concentrated fertilizer mixture and of the separate ingredients

TREATMENT NUMBER	FERTILIZATION	SUPPLEMENT	NUMBER OF SEEDLINGS	SEEDLINGS INJURED Per pot	SEEDLINGS INJURED Average
				per cent	*per cent*
1	Complete	None	10 15	100 100	... 100
2	Complete	Sodium chloride	13 14	100 100	... 100
3	Complete	Potassium chloride	15 13	100 100	... 100
4	Complete	Calcium chloride	13 15	16 13	... 15
5	Complete	Magnesium chloride	13 14	38 29	... 34
6	None	None	13 13	0 0	... 0
7	None	Sodium sulfate	13 10	0 0	... 0
8	None	Potassium sulfate	12 13	0 0	... 0
9	None	Sodium chloride	12 13	0 0	... 0
10	None	Potassium chloride	14 11	0 0	... 0
11	None	Calcium sulfate	12 11	0 0	... 0
12	None	Magnesium sulfate	12 13	0 0	... 0
13	None	Calcium chloride	14 12	0 0	... 0
14	None	Magnesium chloride	13 13	0 0	... 0
15	Diammonium phosphate	None	13 12	100 100	... 100

TABLE 1—*Continued*

TREATMENT NUMBER	FERTILIZATION	SUPPLEMENT	NUMBER OF SEEDLINGS	SEEDLINGS INJURED	
				Per pot	Average
				per cent	*per cent*
16	Diammonium phosphate	Sodium sulfate	13	100	. .
			12	100	100
17	Diammonium phosphate	Potassium sulfate	12	100	. . .
			8	100	100
18	Diammonium phosphate	Sodium chloride	14	100	. .
			13	100	100
19	Diammonium phosphate	Potassium chloride	14	100	. .
			14	100	100
20	Diammonium phosphate	Calcium sulfate	13	70	. . .
			10	50	60
21	Diammonium phosphate	Magnesium sulfate	12	100	. .
			11	75	88
22	Diammonium phosphate	Calcium chloride	15	60	. . .
			10	80	70
23	Diammonium phosphate	Magnesium chloride	12	100	. . .
			13	75	88
24	Potassium nitrate	None	13	16	. . .
			14	7	12
25	Potassium nitrate	Sodium sulfate	7	29	. . .
			13	16	23
26	Potassium nitrate	Potassium sulfate	13	33	. . .
			12	31	32
27	Potassium nitrate	Sodium chloride	12	9	. . .
			9	33	21
28	Potassium nitrate	Potassium chloride	13	70	. . .
			15	13	42
29	Potassium nitrate	Calcium sulfate	13	7	. . .
			15	0	4
30	Potassium nitrate	Magnesium sulfate	12	25	. . .
			13	16	21
31	Potassium nitrate	Calcium chloride	11	9	. . .
			14	43	26

TABLE 1—*Concluded*

TREATMENT NUMBER	FERTILIZATION	SUPPLEMENT	NUMBER OF SEEDLINGS	SEEDLINGS INJURED	
				Per pot	Average
				per cent	*per cent*
32	Potassium nitrate	Magnesium chloride	10	40	...
			12	33	37
33	Potassium chloride	None	12	0	...
			12	0	0
34	Potassium chloride	Sodium sulfate	12	0	...
			12	0	0
35	Potassium chloride	Potassium sulfate	12	0	...
			13	0	0
36	Potassium chloride	Sodium chloride	12	0	...
			12	0	0
37	Potassium chloride	Calcium sulfate	11	0	...
			12	0	0
38	Potassium chloride	Magnesium sulfate	14	0	...
			13	0	0
39	Potassium chloride	Calcium chloride	12	0	...
			10	0	0
40	Potassium chloride	Magnesium chloride	12	8	...
			14	21	15

ium ion if there were no alternate hypotheses suggested by the results of other investigations.

According to Ross, Merz, and Jacob (10), a 1/10 molecular solution of diammonium phosphate has a pH of 8.0 indicating an appreciable degree of hydrolysis with the formation of free ammonia. This is supported by the work of Warren (15), which shows that solid diammonium phosphate has an ammonia vapor pressure of 1.4 mm. at 80°C. The possibility of toxic concentrations of free ammonia being dissociated from the diammonium phosphate in soils of low colloid content is therefore not remote.

On the basis of this hypothesis, it is possible that the protective effect of the added calcium salt might have resulted from a reaction with ammonia, which would be converted to the carbonate by the soil atmosphere, by which the calcium would be precipitated as carbonate and the ammonia remain in solution as the sulfate.

Shive (12) has reported moreover that the phosphate ion is toxic and there

are no good grounds for disregarding the possibility that the beneficial effect of calcium salts may be due to the formation of a relatively insoluble calcium phosphate by reaction with the diammonium phosphate.

As a further complication there is the possibility suggested by the work of Kahlenberg and True (7) and of Hoagland (6) that the hydroxyl ions may be the toxic component.

Toxicity of ammonium salts

In an effort to eliminate as many of these hypotheses as possible, the first step was to determine the toxicity of other salts of ammonia by supplying to each pot equivalent amounts of ammonium from all sources. In this and all subsequent series of pots the basal rate of application was 1 gm. of diammonium phosphate, and the amount of calcium salt used as a supplementary treatment was correspondingly reduced to 0.51 gm. of calcium sulfate or its equivalent.

From the results in table 2 it is distinctly evident that the ammonium ion is not specifically toxic under the conditions of these experiments, but the injury with the monoammonium phosphate rather supports the idea that the phosphate ion or the ammonium combined as phosphate may be moderately toxic. The severity, however, is very much less than that experienced with the diammonium phosphate and it may be that doubling the rate of application of the phosphate ion to maintain the ammonium concentration constant has in this case aggravated a cause of toxicity of minor importance. This assumption is supported by the observation that, with the corrective effect of calcium sulfate, the diammonium phosphate was the less injurious.

The toxicity of phosphates

If the injury from the diammonium phosphate were due to the toxicity of the phosphate ion and the remedial effect of calcium salts were a result of decrease in the phosphate-ion concentration, no benefit should be noted from the use of calcium phosphate as a supplementary treatment. The result of a test of monocalcium phosphate in this capacity is given in table 3, calcium sulfate being included for comparison. Both calcium salts were used in chemically equivalent amounts.

Although the calcium phosphate is not so efficient a corrective of toxicity as is the sulfate it did produce a distinct favorable response. It is obvious from this result that the remedial effect of the calcium salt cannot be due to a reduction of the concentration of toxic phosphate ions and, by inference, it appears doubtful that the toxicity of diammonium phosphate can be due entirely to the high concentration of phosphate.

Some consideration has been given to the possibility that diammonium phosphate is toxic because of the liberation of free ammonia by reaction of the salt with the soil. This could be expected if the phosphate ion were more rapidly absorbed than the ammonium ion. The failure of several attempts to show significant differences in the rates of absorption of these two ions does not, however, offer much support for such a hypothesis.

TABLE 2

Toxicity of ammonium salts

TREATMENT NUMBER	FERTILIZATION	SUPPLEMENT	NUMBER OF SEEDLINGS	INJURY TO SEEDLINGS: Per pot	INJURY TO SEEDLINGS: Average
				per cent	*per cent*
1	Diammonium phosphate	None	9	100	...
			14	100	100
2	Diammonium phosphate	Calcium sulfate	13	15	...
			15	27	21
3	Monoammonium phosphate	None	13	62	...
			15	62	62
4	Monoammonium phosphate	Calcium sulfate	13	54	...
			12	33	44
5	Ammonium nitrate	None	11	0	...
			13	0	0
6	Ammonium nitrate	Calcium sulfate	14	0	...
			10	0	0
7	Ammonium sulfate	None	12	0	...
			13	0	0
8	Ammonium sulfate	Calcium sulfate	15	0	...
			14	0	0
9	Ammonium chloride	None	11	0	...
			13	0	0
10	Ammonium chloride	Calcium sulfate	13	0	...
			14	0	0

TABLE 3

Antagonism between diammonium phosphate and monocalcium phosphate

TREATMENT NUMBER	FERTILIZATION	SUPPLEMENT	NUMBER OF SEEDLINGS	SEEDLINGS INJURED: Per pot	SEEDLINGS INJURED: Average
				per cent	*per cent*
1	Diammonium phosphate	None	9	100	...
			14	100	100
2	Diammonium phosphate	Monocalcium phosphate	14	62	...
			13	62	62
3	Diammonium phosphate	Calcium sulfate	13	15	..
			15	27	21

Free ammonia as the toxic component

The remaining hypotheses, that the hydroxyl ion or that free ammonia might be the toxic factors, were investigated through the use of calcium carbonate as a supplementary treatment at a rate equivalent to the applications of calcium sulfate. The carbonate should not greatly modify the hydroxyl-ion concentration of the solution of diammonium phosphate nor would it neutralize any free ammonia. On the other hand, because of its comparative insolubility it might be ineffective or by the liberation of free ammonia it might increase the toxicity. To cover this latter contingency, the effect of calcium carbonate and calcium sulfate on the toxicity of ammonium hydroxide was also studied. Ammonium hydroxide was distributed in 10 ml. of solution at a rate equivalent to one-half of the total ammonium content of 1 gm. of diammonium phosphate. This ratio was adopted to provide as nearly as possible a quantity of free ammonia comparable with that of the hydrolyzable secondary ammonium radical of the diammonium phosphate.

There are numerous limitations to such a comparison. The initial concentration of free ammonia added as hydroxide would be much greater than the equilibrium concentration of free ammonia derived from the diammonium phosphate. In contact with the soil, however, the concentration of free ammonia from the added hydroxide would rapidly be reduced by adsorption whereas that produced by the hydrolysis of the phosphate should be more nearly constant. Another indeterminate factor is the effect of carbonation of the free ammonia from both sources by contact with the soil atmosphere. The limitations imposed by these variable factors do not, however, entirely preclude a comparison of these two materials and the remedial effect of calcium compounds.

The failure of the calcium carbonate to correct the injury from the diammonium phosphate (table 4) does not controvert the fundamental property of calcium in this respect because of the possibility already suggested that the calcium carbonate might increase the concentration of free ammonia without correspondingly increasing the concentration of calcium available for antagonism.

The similarity in the degree of injury from ammonium hydroxide and from diammonium phosphate does not prove that the causes are qualitatively alike although the effectiveness of calcium sulfate as a corrective of the ammonium hydroxide toxicity gives strong support to that conclusion. The slight but probably significant decrease in the extent of injury from ammonium hydroxide when supplemented by calcium carbonate eliminates the probability that a reduction in the concentration of hydroxyl ions was the sole cause of the previously noted remedial effect of the calcium salts (plate 3).

This result with calcium carbonate and ammonium hydroxide strongly indicates that the calcium has a direct physiological effect independent of any chemical reaction in the medium. There is the possibility however that the greater efficiency of the sulfate is not due entirely to a more intensive antagon-

ism but to an additive effect of a chemical reaction, probably the formation of the non-toxic ammonium sulfate.

In practice, fertilization with diammonium phosphate or other materials productive of free ammonia may not be injurious on highly absorptive soils or when the fertilizer is applied long enough in advance of planting to provide for complete absorption. Under other conditions, the use of gypsum as a supplement may constitute an effective means of control. Ground limestone will probably not serve the same purpose.

This type of fertilizer injury constitutes a problem only with germinating and seedling plants and it is probable that the ultimate effect on the crop would depend on the nature of the rooting systems of the plants fertilized. Taprooted

TABLE 4

The effect of calcium carbonate on free ammonia injury

TREATMENT NUMBER	FERTILIZATION	SUPPLEMENT	NUMBER OF SEEDLINGS	INJURY TO SEEDLINGS	
				Per pot	Average
				per cent	*per cent*
1	Diammonium phosphate	None	15	100	...
			13	100	100
2	Diammonium phosphate	Calcium carbonate	9	90	...
			11	100	95
3	Ammonium hydroxide	None	12	100	...
			14	93	97
4	Ammonium hydroxide	Calcium sulfate	11	36	...
			13	69	51
5	Ammonium hydroxide	Calcium carbonate	9	77	...
			14	78	78

plants would naturally be most subject to damage but this might be corrected by the later development of lateral roots.

Although the method of use of the fertilizer would also govern the probability of injury it would seem best to correct as far as possible all of the toxic properties that are recognized, leaving the placement of the fertilizer to control only the otherwise unavoidable causes of injury.

SUMMARY

In a pot culture experiment with a sandy soil of low absorptive capacity a mixture of C. P. diammonium phosphate, potassium nitrate, and potassium chloride was toxic to cotton seedlings. The cause of injury was related to some property of the diammonium phosphate. The rate of application of the mix-

ture was equivalent to 96 pounds to the acre, in the drill, of a fertilizer analyzing 17.5 per cent nitrogen, 35 per cent phosphoric acid, and 17.5 per cent potash.

The free ammonia formed by the hydrolysis of diammonium phosphate was apparently the most toxic component.

No injury was observed from the use of ammonium as the sulfate, chloride, or nitrate, nor did the alkalinity of the diammonium phosphate appear to contribute to the injurious effect.

Calcium salts were antagonistic to ammonia toxicity, calcium sulfate and chloride being more effective in this regard than calcium carbonate and phosphate. Magnesium salts exhibited the same effect but they functioned less efficiently than did the corresponding calcium salts.

The conversion of free ammonia to carbonate in the soil and its partial neutralization by reaction with the calcium salts could account for the greater part of the beneficial effect of the calcium salts but there was some evidence that physiological antagonism was also a factor. It is not impossible that both of these may contribute to the favorable result.

Monoammonium phosphate alone produced a lesser degree of injury than did diammonium phosphate. This relation was reversed when both were supplemented by admixture with calcium sulfate.

The practical possibilities of gypsum as a supplement to all fertilizers or other soil treatments supplying free ammonia to soils in toxic concentrations is indicated by the work.

REFERENCES

(1) Beaumont, A. B. 1925 The use of concentrated fertilizers. *Amer. Fert.* 62: 23–24.

(2) Beaumont, A. B. 1927 A burning question. *Better Crops* 7: 12.

(3) Darwin, Chas. 1882 On the action of carbonate of ammonia on the roots of certain plants. *Linn. Soc.* [London] March 16. [Reference quoted from (4).]

(4) Ehrenberg, P. 1907 Die Bewegung des Ammoniakstickstoffes in der Natur. *Mitt. Landw. Inst. Breslau* 4: 47–300.

(5) Hansteen-Cranner, B. 1914 Über das Verhalten der Kulturpflanzen zu den Bodensalzen. III. Beiträge zur Biochemie und Physiologie der Zellwand lebender Zelle. *Jahrb. Wiss. Bot.* 53: 536–598.

(6) Hoagland, D. R. The effect of hydrogen and hydroxyl ion concentration on the growth of barley seedlings. *Soil Sci.* 3: 547–559.

(7) Kahlenberg, L., and True, R. H. 1896 On the toxic action of dissolved salts and their electrolytic dissociation. *Bot. Gaz.* 22: 81–124.

(8) Osterhout, W. J. V. 1916 A dynamical theory of antagonism. *Proc. Amer. Phil. Soc.* 55: 533–553.

(9) Raber, O. 1923 Permeability of the cell to electrolytes. *Bot. Gaz.* 75: 298–308.

(10) Ross, Wm. H., Merz, A. R., and Jacob, K. D. 1929 Preparation and properties of the ammonium phosphates. *Indus. and Engin. Chem.* 21: 286–293.

(11) Russell, E. J., and Petherbridge, F. R. 1913 On the growth of plants in partially sterilized soils. *Jour. Agr. Sci.* 5: 248–287.

(12) Shive, J. W. 1916 A study of the toxic influence of phosphate salts on soybean seedlings. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1916: 449–54.

(13) Skinner, J. J. 1929 New fertilizer materials and their uses. *N. C. Agr. Exp. Sta. Agron. Cir.* 22.

(14) SKINNER, J. J., WILLIAMS, C. B., AND MANN, H. B. 1929 Effects of synthetic nitrogen and concentrated fertilizers on cotton and sweet potatoes. *N. C. Agr. Exp. Sta. Bul.* 266.

(15) WARREN, T. E. 1927 Dissociation pressures of the ammonium orthophosphates. *Jour. Amer. Chem. Soc.* 49: 1904–1908.

PLATE 1

Type of Injury to Cotton Seedlings and Degree of Control as a Result of Complete Fertilizer Supplemented with Other Treatments

In this plate and in plates 2 and 3, all the germinated seedlings from each of duplicate pots are shown on either side of the reference number. The dark line indicates the plane of fertilizer application.

Fig. 1. Complete fertilizer treatment no. 1 (table 1).

	gm.
Diammonium phosphate	0.98
Potassium nitrate	0.40
Potassium chloride	0.12

Fig. 2. Complete fertilizer treatment no. 4 (table 1).

	gm.
Complete fertilizer	1.5
Calcium chloride	0.59

FIG. 1

FIG. 2

PLATE 2

Effect of Diammonium Phosphate on Growth of Cotton Seedlings

Fig. 1. Diammonium phosphate (1 gm.) treatment no. 1 (table 3).

Fig. 2. Diammonium phosphate treatment no. 3 (table 3).

	gm.
Diammonium phosphate	1
Calcium sulfate	0.51

Fig. 3. Diammonium phosphate treatment no. 2 (table 3).

	gm.
Diammonium phosphate	1
Monocalcium phosphate	0.75

PLATE 2

FIG. 1

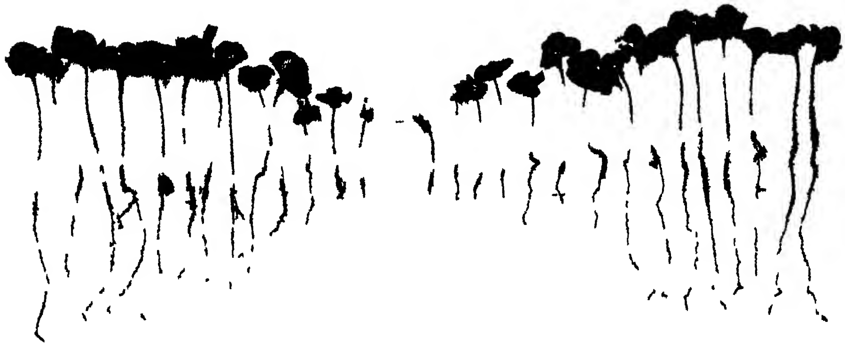


FIG. 2

FIG. 3

PLATE 3

EFFECT OF CALCIUM SALTS ON AMMONIUM HYDROXIDE INJURY TO COTTON SEEDLING

FIG. 1. Ammonium hydroxide (0.13 gm.) treatment no. 3 (table 4).

FIG. 2. Ammonium hydroxide treatment no. 4 (table 4).

	gm.
Ammonium hydroxide	0.13
Calcium sulfate	0.51

FIG. 3. Ammonium hydroxide treatment no. 5 (table 4).

	gm.
Ammonium hydroxide	0.13
Calcium carbonate	0.29

Fig. 1

Fig. 2

Fig. 3

THE NATURE OF THE BASE-EXCHANGE MATERIAL OF BENTONITE, SOILS, AND ZEOLITES, AS REVEALED BY CHEMICAL INVESTIGATION AND X-RAY ANALYSIS

W. P. KELLEY, W. H. DORE AND S. M. BROWN

University of California[1]

Received for publication April 14, 1930

It is well established that the replacement of one cation by another produces significant changes in the chemical, physical, and plant nutritional properties of the soil. Various investigators have recognized that soils vary in their content of replaceable cations. "Base-exchange capacity" is the term employed by many soil investigators to denote the total content of all exchangeable cations. Hissink's T value represents a similar conception.

J. T. Way concluded in 1852 that the base-exchange substances of soils are of two kinds, namely, organic and inorganic. The discussion presented in this paper is concerned entirely with the inorganic constituents. Way suggested that the inorganic base-exchange constituents of soils belong to the alumino silicates, and that they are analogous to the zeolites. This view is still held by many soil investigators both in Europe and in America. On the other hand, Van Bemmelen concluded more than 40 years ago that the replaceable bases are held by the soil not as ordinary chemical compounds, but that they are adsorbed on the surface of the colloidal particles of the soil. The adsorption hypothesis, with perhaps slight modification, is still held by many European investigators. The term "adsorption compounds" is often used to denote the base-exchange material of the soil.

In connection with the study of the base-exchange property of soils, we have devoted considerable attention to a natural clay known as bentonite. It is now well established that this clay is remarkably similar to the soil in so far as its base-exchange property is concerned. By subjecting the crude clay material to the process of sedimentation, it is possible to separate the base-exchange substance from most of the various impurities which are commonly associated with it.

Many of the bentonitic clays are highly colloidal and possess pronounced base-exchange power. Ross and Shannon (5) have shown that the dominant and most important constituent of certain types of bentonite is the micaceous crystalline mineral, montomorillonite. As is well known by colloid chemists,

[1] Paper No. 218, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California. Joint contribution from Laboratory of Agricultural Chemistry, Riverside, and Laboratory of Plant Nutrition, Berkeley.

the fact that a given material is crystalline does not preclude its being colloidal, for there are many known examples of crystalline materials which possess pronounced colloidal properties. As used by chemists the term "colloidal" refers primarily to the size of the particles and the fact that the particles will remain in suspension more or less indefinitely, and not to the shape, atomic structure, or the chemical composition of the particles. Obviously, if a colloidal material is crystalline, the individual crystals must necessarily be extremely small. Wherry (7) has suggested that the tabular crystals of bentonitic clays are microscopic in breadth but of colloidal thickness.

As stated already the bentonitic clays possess pronounced base-exchange property. We have found, however, that the purified substances separated from the crude clays of different sources, differ both in their total content of replaceable bases and in the relative ratios of different replaceable bases. All of the bentonites that we have studied contain more or less replaceable magnesium. The deposits which occur in certain localities contain considerable replaceable calcium. Other samples contain much replaceable sodium. We have examined one sample which contains considerable replaceable potassium. All of these bentonites contain much magnesium, and one of them considerable potassium, that are not replaceable by ordinary methods. On the other hand, practically all of the calcium and sodium are replaceable.

EFFECT OF GRINDING

Three of the purified bentonites which we have studied were found to be remarkably similar, having, as shown in table 1, a base-exchange capacity of 105 to 110 milliequivalents to every 100 gm. of air-dry material. The base-exchange capacity of the purified bentonites obtained from other sources ranged from 82 to 88 milliequivalents to every 100 gm., whereas still other samples contain about 55 milliequivalents of replaceable bases. Since one type of bentonite that was employed in our investigation was known to contain the crystalline mineral, montmorillonite, it was decided to study the effects produced by grinding these materials in a ball mill.

In the first experiment a sample of calcium-saturated bentonite, containing 106 milliequivalents of replaceable calcium, was placed in a ball mill and ground for several hours. The material was then subjected to a determination of the replaceable bases. The results showed that the grinding process materially increased the replaceable magnesium, but did not appreciably affect the replaceability of other elements. As is shown in table 2, grinding for 72 hours brought about a very marked increase in the replaceable magnesium.

The bowl of the ball mill used in this investigation was porcelain lined and the balls were composed of high-carbon-chromesteel, $\frac{3}{8}$ inch and $\frac{1}{2}$ inch in diameter. The flint pebbles, commonly employed in ball mill grinding, are not satisfactory for this type of experiment, because they undergo abrasion to such an extent as substantially to contaminate the experimental material. We have not attempted to determine the most efficient method of grinding these

materials. All of the samples were air dried. It was noted that the colloidal materials tended to cake on the walls of the ball mill and the surface of the balls. It is probable that the particles of colloids of this type will be more effectively ground if the sample is partially dehydrated. In other experiments some indication was found that grinding affects the Ca-saturated material to a greater extent than the Na-saturated form. This is probably due to differences in absorbed water.

As has been stated, the sample used in the first experiment was approximately calcium saturated before being subjected to grinding. The calcium

TABLE 1

*Replaceable bases of bentonites**

LABORATORY NUMBER	LOCALITY	MILLIEQUIVALENTS PER 100 GM.				
		Ca	Mg.	K	Na	Total
1	Death Valley, California	32	34	7	33	106
2	San Diego, California	34	54	4	18	110
3	Osage, Wyoming	7	8	5	66	86
4	Merritt, British Columbia	36	15	3	4	58
5	Rosedale, Alberta	26	11	5	46	88
6	Princeton, British Columbia	45	8	0	0	53
7	Goldfield, Nevada	27	28	6	44	105
8	Medicine Bow, Wyoming	18	20	4	40	82

* The analyses were made on products separated from the crude bentonites by the process of repeated sedimentation in alcohol.

TABLE 2

Effect of grinding bentonite No. 7

	MILLIEQUIVALENTS REPLACEABLE BASES				
	Ca	Mg	K	Na	Total
Unground sample*..............................	106	0	0	0	106
Ground 30 hours	107	68	Trace	Trace	175
Ground 72 hours..............................	106	222	Trace	Trace	328

* This sample was saturated with calcium before the experiments were made.

saturation was effected by prolonged leaching with a neutral solution of normal calcium chloride, the occluded calcium chloride being then removed by leaching with water. After being thus saturated with calcium the sample contained 4.60 per cent total MgO, which, as stated already, was non-replaceable before grinding. After the sample had been ground for 72 hours, a high percentage of the total magnesium was replaceable. If the sample is ground sufficiently thoroughly, probably all of the magnesium will be replaceable.

Bentonites from other sources were also investigated. One of these, bentonite no. 2, was saturated with calcium before it was subjected to grinding.

The other sample, no. 3, was separated by sedimentation in methyl alcohol. The material thus obtained contained the replaceable bases originally present in the crude clay. The results are shown in table 3. It will be noted that grinding produced much the same effect with bentonite no. 2 as with bentonite no. 7, reported in table 2. On the other hand, bentonite no. 3 was affected to a lesser degree. The chief replaceable base of this last named sample, before it was ground, was sodium, and its total content of all replaceable bases was approximately 85 milliequivalents to every 100 gm. It will be noted that again the only base whose replaceability was affected by the grinding was magnesium.

Grinding experiments were also made with a sample of the calcium-saturated material separated from the so-called potash bentonite (5), and a similar experiment was made on a sample of beidellite. According to Ross and Shannon, beidellite belongs to the bentonitic clays and is related to the montmorillonite bentonites. The results are reported in table 4.

TABLE 3

*Effect of grinding bentonites**

	MILLIEQUIVALENTS REPLACEABLE BASES				
	Ca	Mg	K	Na	Total
Bentonite no. 2, unground	108.0	0	0	0	108.0
Bentonite no. 2, ground 72 hours	106.0	220.5	Trace	Trace	326.5
Bentonite no. 3, unground	6.6	12.6	3.7	61.7	84.6
Bentonite no. 3, ground 24 hours	7.8	44.1	4.1	65.3	121.3

* Bentonite no. 2 was saturated with calcium before the experiments were made. Untreated bentonite no. 3 was used.

It will be noted that before these materials were ground their total replaceable base content was considerably less than that of the bentonites already discussed. Grinding, however, produced marked effects on the replaceable-base content. In the case of the potash bentonite the replaceable magnesium and potassium were both markedly increased. The replaceable potassium of the beidellite was also increased to a considerable extent. The magnesium, however, was affected to a still greater extent. On the other hand, the replaceable calcium and sodium were but slightly affected by the grinding.

Effect of grinding soil colloids

The marked effects on the replaceable bases produced by grinding the colloidal samples of bentonite and beidellite suggested similar experiments with soil colloids. The method used in separating the colloidal material from the soil mass was essentially as follows: Several hundred grams of air-dry soil was leached with a solution of sodium chloride in order to replace the natural bases with sodium and thus facilitate the dispersion of the colloidal material. The soil

was then suspended in a relatively large volume of water, and the material remaining in suspension after standing 24 hours was syphoned off. The residue was again shaken with water and the process of sedimentation and syphoning was repeated several times. The colloidal suspensions thus obtained were coagulated with calcium chloride and re-treated several times with fresh solutions of calcium chloride. After the excess of calcium chloride was leached out with distilled water the organic constituents were removed, either by direct oxidation with hydrogen peroxide, or by first extracting with 4 per cent ammonia and then oxidizing with hydrogen peroxide. When the organic constituents had been removed, as was indicated by a carbon determination, the inorganic colloidal material was thoroughly leached with normal calcium acetate in order to effect complete calcium saturation. The soluble calcium salt was leached out with methyl alcohol and the sample was dried at a low temperature. The material was then reduced to a fine powder by careful crushing in a hand mortar.

TABLE 4

*Effect of grinding potash bentonite and beidellite**

	MILLIEQUIVALENTS REPLACEABLE BASES				
	Ca	Mg	K	Na	Total
Potash bentonite, unground	31.8	2.0	0.9	0	34.7
Potash bentonite, ground 30 hours	32.3	38.4	29.0	2.3	102.0
Beidellite, unground	47.0	4.2	Trace	Trace	51.2
Beidellite, ground 3 days	55.0	59.0	18.7	1.7	134.4

* These minerals were obtained from C. S. Ross of the U. S. Geological Survey. The samples were saturated with calcium before being ground.

Five different soil colloids were investigated by the grinding method. The results are recorded in table 5. It will be noted that the effect of grinding these soil colloids was remarkably similar to that produced on the bentonites. The replaceable bases of soil colloid no. 7083, which was separated from a California soil, were most markedly affected, the total replaceable base content being increased from approximately 65 milliequivalents to 205 milliequivalents. It will be noted that with this soil colloid magnesium was the chief base whose replaceability was affected, more than 100 milliequivalents of magnesium having been made replaceable as a result of grinding. Approximately 29 milliequivalents of potassium were also made replaceable. Another soil colloid from California, no. 431, was also markedly affected by grinding, and in this case magnesium was again the chief base whose replaceability was increased. The replaceability of the potassium was also affected, but to a smaller degree than that of magnesium. The replaceability of calcium was not materially affected by grinding either of these soil colloids.

Similar results were obtained with colloids 3232 and 7680. The former was

separated from a glacial drift soil of Indiana and the latter from a limestone soil of central Tennessee. It will be noted that the effect of grinding was less marked with these samples than with the soil colloids from the semiarid region of California, but again the elements that were most markedly affected were magnesium and potassium. With these samples grinding also produced very little effect upon the replaceability of the calcium. The colloid from a highly acid soil of the Cecil series is especially interesting in that the effect of grinding was most marked with the H ion. The calcium-saturated form of this colloid was approximately neutral, but, after being ground, its pH was found to be 5.8. It then contained 60 milliequivalents of replaceable H ions. With this soil colloid the bases whose replaceabilities were increased to the greatest extent by grinding were again magnesium and potassium, but the increases were less marked than in the case of the previously mentioned soil colloids.

Tables 2, 3, 4, and 5, indicate that the soil colloids are remarkably similar to the bentonitic clays, in so far as the effect of grinding is concerned. With the soil colloids from California, 431 and 7083, magnesium was the base most markedly affected, whereas the effect of grinding the colloids from the soils of the more humid parts of the United States was distributed approximately equally between magnesium and potassium. In no case was the replaceability of calcium markedly affected by grinding.

It will be noted that the total content of replaceable bases held by the unground samples of soil colloids 431, 7083, 3232, and 7680, is of the same general magnitude as that of the bentonites 4 and 6, potash bentonite, and beidellite. Moreover, grinding increased the content of replaceable bases of these materials similarly.

The total bases of these soil colloids are reported in table 6. The data are expressed as milliequivalents for each 100 gm. for convenience of comparison with the replaceable bases. It will be seen that, with the exception of colloid 7680, practically all of the calcium contained by these soil colloids is replaceable. Total analyses of the bentonites showed that practically all of their calcium is also replaceable. It will be noted that although the potassium in the unground samples of these soil colloids was not replaceable practically all of it was made replaceable by grinding. The same is true as regards the magnesium of soil colloid 431. On the other hand, much of the magnesium in the other soil colloids still remained in a non-replaceable form after they had been subjected to grinding for the periods employed in these experiments. In view of the result obtained by grinding the sample of bentonite for different periods of time, it is possible that more drastic grinding of the soil colloids would bring about a still further increase in the replaceable magnesium. All of these soil colloids were quite low in total sodium.

Thus it is shown that the chief effect of grinding these soil colloids was, first, to increase the replaceability of their magnesium to a marked extent, and, secondly, to render the potassium replaceable. The results are, therefore, markedly similar to those obtained with the bentonites. The preceding results

strongly suggest that the base-exchange constituents of both the soil colloids and the highly colloidal bentonites are crystalline and that the replaceable bases are contained within the interior of the crystals as well as on the exterior.

X-RAY INVESTIGATIONS

In order to obtain further information concerning the nature of the base-exchange materials, we have made studies on a number of samples of bentonites and soil colloids by the method of X-ray crystal analysis.

TABLE 5

*Effect of grinding soil colloids**

	MILLIEQUIVALENTS REPLACEABLE BASES				
	Ca	Mg	K	Na	Total
Soil colloid 7083, unground	58.1	6.1	0.3	1.1	65.6
Soil colloid 7083, ground 30 hours	60.4	109.3	29.0	5.4	204.9
Soil colloid 431, unground	54.7	2.5	0	0	57.2
Soil colloid 431, ground 30 hours	59.9	71.2	25.3	0	156.4
Soil colloid 3232, unground	54.0	2.7	1.0	1.4	59.1
Soil colloid 3232, ground 72 hours	57.3	21.0	19.3	13.6	111.2
Soil colloid 7680, unground	32.8	2.3	0	1.0	36.1
Soil colloid 7680, ground 30 hours	41.7	27.3	26.0	0	95.0
Soil colloid 11954, unground	14.2	0	0.2	3.9	18.3
Soil colloid 11954, ground 30 hours	14.0	6.8	7.3	6.0	34.1

*No. 431, Ramona clay loam, taken near La Habra, California.
No. 7083, Dublin clay adobe, taken near Gilroy, California.
No. 3232, Glacial drift soil from Indiana.
No. 7680, Limestone soil, central Tennessee.
No. 11954, Cecil clay subsoil, Alabama.
All samples were saturated with Ca before grinding.

TABLE 6

Fusion analysis of soil colloids

	MILLIEQUIVALENTS PER 100 GM.			
	Ca	Mg	K	Na
431	58	68	20	11
7083	80	142	22	22
3232	60	38	25	15
7680	101	155	33	22
11954	14	20	7	7

The method depends upon the following principles: In crystalline substances, the constituent atoms are located at regularly spaced distances in the various directions, thus forming a regular three-dimensional space lattice. The regularly spaced atoms fall into planes and these planes are also regularly spaced. Because the interplanar spacings are of about the same order of magnitude

as the wave lengths of X-rays, the planes of atoms in a crystal are capable of acting as a diffraction grating for X-rays, in much the same way that the closely ruled lines on a glass plate diffract the waves of visible light. In the case of visible light, the wave length may be calculated from data which include knowledge of the spacings separating the lines which compose the grating. Similar application is made of the diffraction principle in X-ray crystal analysis, but in this case it is the wave length of monochromatic X-rays which must be known, while the quantity corresponding to the grating spacing is to be determined. This unknown quantity is the distance separating the planes of atoms in the crystal; it is the key to the whole problem of crystal structure.

When x-rays strike the atoms of a crystalline substance the rays are scattered in all directions, and in general these scattered rays are not intense enough to produce more than a slight fogging effect on a photographic film. If, on the other hand, a number of these scattered rays are deflected in such a manner that the deflected rays are in phase, their combined intensities will be sufficient to register on a stuitably placed film. It can be shown by simple trigonometry (1) that X-rays are deflected in phase from the atomic planes of a crystal when the relationship between the wave length of the X-rays (λ), the interplanar spacing (d) and the glancing angle Θ between the incident beam and the reflect ing plane is described by the equation:

$$n \lambda = 2\, d \operatorname{Sin} \Theta$$

in which n is a simple integer representing the order of reflection. Accordingly, if the wave length, λ, be known and the glancing angle, Θ, be observable, it becomes possible to calculate d/n, that is, the ratio of the interplanar spacing to the order of reflection. Since n is a small integer and it is usually possible to decide whether it is 1, 2, or 3, etc., the value of the interplanar spacing is readily obtained.

In practice a single crystal, or a tube of the powdered crystals, is placed in the path of an X-ray beam, and the undeflected beam, as well as the various beams which are deflected in accordance with the foregoing equation, are intercepted and registered on a suitably placed photographic film or plate. From measurements on the film, the angular displacement of the beam ($= 2\,\Theta$) may be determined. If a single crystal is used with monochromatic X-rays, it is necessary to make a number of exposures with the crystal in different positions in order to obtain the various interplanar spacings, since for any one position we have fixed values for λ and Θ and consequently can have only one value for d/n. If, on the other hand, the sample is in the form of a fine powder, it consists of a large number of minute crystals in random arrangement, and some of the crystalline fragments, according to the law of probability, will have the required position with respect to the X-ray beam for any given interplanar spacing. Collectively, therefore, the particles will produce reflections in phase corresponding to all interplanar spacings present in the crystals.

It follows, then, that when powdered crystalline material is examined in an

X-ray diffraction apparatus, a series of lines is recorded on the photographic film, and that these lines correspond to the interplanar spacings of the material. This series of lines is known as the diffraction pattern and is characteristic of the substance which produces it. The production of a pattern is direct evidence that the substance is crystalline. Under favorable conditions the pattern may be interpreted so as to give a more or less complete picture of the structure of the crystalline substance.

The replaceable-base materials were examined in the form of fine powders that were passed through a wire sieve having 200 meshes to the linear inch. The samples were packed into thin walled glass tubes having a diameter of about 1 mm., and exposed in the X-ray diffraction apparatus supplied by the General Electric Company. A Coolidge tube with a molybdenum target was used as the source of X-rays and the X-rays were passed through a zirconium oxide screen so as to give monochromatic X-rays with a wave length of 0.712 Ångstrom units. The photographic film was placed on an arc of 8-inch radius, at the center of which was located the tube containing the specimen. The incident beam passed through a narrow slit (0.02 inch x 0.5 inch) and images of this slit were produced on the film by the undeflected beam and by the various deflected beams. By measuring the distance of the various lines from the "zero line," representing the intercept of the undeflected beam, values are obtained which are proportional to the angular displacement in radians.

Samples of bentonites from nine different sources, soil colloids from six different sources, and one sample each of beidellite and halloysite were examined by the powder method. All of these were examined in their original unground condition, some of them after prolonged grinding, some also after saturation with various replacing bases, and one after being heated to various temperatures.

QUALITATIVE REVIEW OF RESULTS

All of these substances showed distinct X-ray diffraction patterns, indicating that they are not amorphous, as has been commonly assumed, but, on the contrary, that they possess definitely crystalline structures. From a merely qualitative inspection it is apparent that there is a considerable similarity between all of the X-ray photographs, certain prominent lines being shown in all of the films (pl. 1 and 2). This suggests that all of these substances have some sort of a common structural basis. On the other hand, the films also show certain differences, both in the lines that are present and in the relative intensities of the lines. This suggests that within the common structural organization, there are certain atoms which, although fitting into the fundamental structure, are not essential to the framework and which give rise to additional reflecting planes that differ in the various samples.

The samples after prolonged grinding in a ball mill (usually 48 to 72 hours) gave X-ray patterns that were less distinct than those from the corresponding unground samples (pl. 3). In certain cases prolonged grinding apparently

produced no change in the pattern, although in some instances the lines almost completely disappeared, but usually the change was confined to only one or two lines in the pattern. The disappearance of a line caused by grinding was invariably accompanied by a darkening of that area of the film which corresponded to the position occupied by the missing line. This darkening effect indicates a broad scattering of the X-rays in the region involved, and signifies a partial breaking down of the crystalline material to an unorganized (i.e., an amorphous) condition. In every case the films of the ground material showed distinct increases in the fog effect, indicating that the grinding reduced more or less of the crystalline material to an amorphous condition.

A series of films was obtained representing bentonite 7 saturated with various replacing bases. All of the patterns were approximately alike (pl. 4, fig.1). A similar series of patterns for soil colloid 431 saturated with calcium, sodium, potassium, ammonium, magnesium, and copper showed no significant differences (pl. 4, fig. 2). That the substitution of one base for another failed to affect the X-ray films was probably because the base replacement, brought about by the treatment of the samples, was limited chiefly to the atoms located on or near the surface of the crystals. Apparently the interiors of the crystals were not affected by this treatment.

X-ray examination was made of four portions of bentonite 7 which had been heated to 350°, 450°, 600°, and 750°C., respectively (pl. 5). The diffraction patterns corresponding to the three lower temperatures were alike and similar to that of the unheated bentonite. The material heated at the highest temperature gave an altogether different and much fainter pattern containing a broad darkened area. This appears to indicate a considerable breaking down to amorphous material at the highest temperature with some alteration to a different type of crystal structure.

The bentonites from eight different sources gave diffraction patterns that are very similar (pl. 1, fig. 1), a number of prominent lines being common to all these films. We will discuss later certain minor differences in these patterns.

The soil colloids gave patterns obviously more complicated than the bentonite patterns, but nevertheless clearly related to them (pl. 1). The beidellite pattern is very similar to the bentonite patterns. The halloysite pattern suggests that of potash bentonite, although it contains fewer lines (pl. 2). These relationships will be discussed more fully in connection with the numerical data.

QUANTITATIVE INTERPRETATION OF RESULTS

As has already been indicated, the numerical values of the interplanar spacings may be obtained from measurements on the films. The films obtained from the various bentonites and soil colloids have been measured and the values of the interplanar spacings are given in tables 7 and 8. These values are expressed in Ångstrom units and opposite each value is an approximate indica-

TABLE 7

*Interplanar spacings of bentonite samples**

NO. 1	NO. 2	NO 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	POTASH BENTONITE	BEIDELLITE	HALLOYSITE	CALCULATED SPACINGS FOR 9.0 Å UNIT CUBE	CORRESPONDING CRYSTAL-LOGRAPHIC INDEXES
5.20 w	5.10 w	....	5.10 tr?	5.10 w	....	5.20 w	5.10 w	5.00 w	....	....	5.19	(111)
4.50 vs	4.50 vs	4.50 vs	4.50 vs	4.50 vs	4.50 vs	4.50 vs	4.50 vs	4.50 vs	4.50 vs	4.45 vs	4.50	(100) [2]
....	....	....	4.10 vs	....	4.10 vvs	....	4.00 w	....	....	....	4.01	(120)
....	....	3.30 m	....	....	....	....	....	3.65 m	....	3.65 m	3.67	(112)
....	....	3.16 w	....	3.20 m	....	3.10 m	3.34 m	3.30 ?	3.30 ?	....	3.18	(110) [2]
3.04 m	3.03 m	....	3.08 m	....	....	....	3.03 w	2.90 ?	....	....	3.00	(100) [3]
....	....	....	....	....	2.86 vvw	....	....	....	....	....	2.84	(130)
{2.57 vs	{2.58 vs	{2.60 s	{2.58 s	{2.58 vs	{2.58 s	{2.62 s	{2.58 vs	2.56 vs	{2.60 s	{2.60 m	2.60	(111) [2]
{2.51	{2.48	{2.50	{2.45	{2.52	{2.45	{2.52	{2.45	....	{2.52	{2.35	2.49	(230)
....	....	....	....	....	....	{2.28 w	....	2.38 s	....	....	2.40	(123)
....	2.24 tr	2.25 tr	2.24 ?	2.24 vvw	....	{2.24	....	....	2.24 vw	....	2.25	(100) [4]
2.20 vvw	....	....	....	....	....	....	....	2.22 vw	....	....	2.19	(140)
....	....	....	....	....	....	....	....	2.00 ?	....	....	2.00	(120) [2]
....	....	....	....	....	....	....	....	....	....	....	1.83	(112) [2]
....	....	....	....	....	....	....	....	....	....	....	{1.73	(111) [3]
1.69 w	1.70 m	1.69 m	1.69 m	1.69 m	1.70 m	1.71 m	1.69 m	1.70 vw	1.70 m	1.69 w	{1.67	(234)
....	....	....	....	....	1.62 vw	....	....	1.63 w	....	....	1.64	(125)
....	....	....	....	....	....	....	....	....	....	....	1.54	(334)
1.50 vs	1.50 vs	1.50 vs	1.50 vs	1.50 vs	1.50 vs	1.51 vs	1.50 vs	1.50 vs	1.50 vs	1.48 s	1.50	(100) [6], (122) [2]
....	....	1.38 vvw	....	....	....	....	1.37 vw	....	....	....	1.37	(335)
1.29 m	1.29 m	1.29 m	1.29 m	1.29 m	1.29 m	1.30 m	1.29 m	1.30 m	1.30 m	1.29 vw	1.30; 1.29	(111) [4]; (100) [7], (236)
1.24 w	1.25 w	1.24 w	1.25 w	1.24 w	1.24 w	1.25 w	1.24 w	1.24 w	1.24 w	1.24 vw	1.245	(230) [2]

* Interplanar spacings expressed in Ångstrom units. Symbols designate relative intensity of the corresponding lines. vvs = very very strong; vs = very strong; s = strong; m = medium; w = weak; vvw = very very weak; tr = trace; ? means line questionable. Bracketed values indicate a broad line which may be a composite of two or more superimposed lines. Figures in square brackets designate orders of reflection.

tion of the intensity of the line. The last two columns in each table are concerned with the interpretation of these data and will be referred to presently.

The general appearance of the patterns suggested that the structure might be a cubic lattice. In order to test this point quantitatively, a number of the spacing values were plotted and compared with charts, according to the method of Hull and Davey as outlined by Wychoff (8). Most of the values thus plotted agree with a cubic lattice based on a unit cube having an edge 9.0 Ångstrom units in length. Calculations were made of the interplanar spacings of the various reflecting planes in a theoretical cube of 9.0 Å. edge, and a list was prepared of all possible spacings between 9.0 Å. and 1.24 Å.

The last two columns of each table give the significant calculated values, together with their corresponding crystallographic indexes (Miller indexes). It will be seen that, with but few exceptions, the observed interplanar spacings agree closely with interplanar spacings calculated from the theoretical cube. Moreover, the observed relative intensities of the lines are similar to the expected intensities from the theoretical cube. From this agreement it is concluded that all of the substances examined have structures related to a cubic lattice with a unit cube having a 9 Å. edge. As some of the materials were obviously heterogeneous, the few lines which do not correspond to any spacings in the theoretical lattice may be ascribed to the presence of foreign material.

It will be observed that the bentonite patterns contain in general a smaller number of lines than the soil colloids, 9 to 14 lines being obtained in a bentonite pattern and 12 to 19 lines in a soil colloid pattern. Altogether 21 different lines are found in the bentonite patterns. All but one of these (the 3.30 to 3.34 Å. spacing) agree with spacings calculated on the theoretical cube. The soil colloid patterns contain a total of 30 different lines; five of these (7.40 Å., 5.80 Å., 4.30 Å., 3.80 Å., and 3.35 Å.) cannot be correlated with any calculated spacings of the theoretical cube, but the other spacings are in satisfactory agreement.

On the basis of the similarity of the crystallographic indexes, the various bentonite patterns may be grouped into classes having structures of varying complexity. The classes merge into one another somewhat and their separation is necessarily inexact and arbitrary. Nevertheless, the patterns fall distinctly into about three classes.

The simplest class includes the patterns of bentonites 1, 2, 7. The prominent lines of these patterns correspond to the following crystallographic indexes: (100), second, fourth, sixth, and seventh orders; (110), second order; (111), second and fourth orders; and second orders of (122) and (230). These observations suggest that the structure of these materials is related to a comparatively simple type of cubic lattice having atoms or groups of atoms at or near the corners of the cube. Chemical analysis shows that these samples have a composition closely approximating that given by Ross and Shannon for montmorillonite.

The next class in order of complexity includes bentonites 3, 4, 5, 6, and 8,

TABLE 8

*Interplanar spacings of soil colloids**

431	5696	7083	7680	3232	11954	CALCULATED SPACINGS FOR 9.0 Å UNIT CUBE	CORRESPONDING CRYSTALLOGRAPHIC INDEXES
....	5.80 ?	....	....	....	7.40 s	—	—
....	....	....	....	....	....	5.19	(111)
4.50 vs	4.50 vs	4.50 vs	4.50 m	4.50 s	4.60 vs	4.50	(100) [2]
....	....	....	4.30 m	4.20 s	4.15	—	—
4.10 m	4.05 s	....	....	....	....	4.01	(120)
....	....	....	....	....	....	—	—
....	3.75 m	....	....	....	3.70 s	3.67	(112)
3.35 s	3.35 s	3.35 s	3.30 vvs	3.35 vvs	3.54	—	—
3.20 s	3.20 vvs	3.20 m	....	....	....	3.18	(110) [2]
....	3.00 w	3.00 w	3.00	....	....	3.00	(100) [3]
2.75 ?	....	....	2.80 vw	....	2.70 m	2.71	(113)
2.64 s	2.64 vs	2.64 vs	2.58 w	2.56 m	2.58 vs	2.60	(111) [2]
2.56	2.54	2.54	....	....	2.50	....	....
2.44 s	2.45 m	2.45 s	2.46 vw	2.45 w	....	2.49	(230)
2.40	....	....	....	....	2.37 s	2.40	(123)
....	2.28 vw	....	2.25 vw	2.28 vw	2.31	2.25	(100) [4]
....	....	....	....	....	2.22 m	2.19	(140)
2.18 w	....	....	....	....	....	2.18	(223)
....	2.13 vw	....	2.13 w	2.12 m	....	2.12	(110) [3]
2.00 w	2.03 vw	....	1.99 vw	1.99 w	2.01 w	2.01	(120) [2]
....	1.82 ?	....	1.83 s	1.82 s	1.84 m	1.83	(112) [2]
....	....	....	....	....	1.72 s	1.73	(111) [3]
1.70 m	1.66 ?	1.70 m	1.67 w	1.67 m	1.68	1.67	(234)
1.54 w	1.54 m	1.54 m	1.54 m	1.54 s	....	1.54	(334)
1.51 s	1.50 s	1.51 s	1.50 s	1.50 s	1.50 vs	1.50	(100) [6], (122) [2]
....	....	....	....	....	1.46 s	1.46	(116)
....	1.38 w	1.38 w	1.38 vs	1.37 vs	....	1.37	(335)
1.30 w	....	1.30 w	....	....	....	1.30	(111) [4]
....	....	....	1.29 w	1.29 w	1.29 w	1.29	(236)
....	....	....	1.25 vw	1.26 vw	1.24 w	1.245	(230) [2]

* For explanations see footnote table 7.

and the sample of beidellite. The diffraction patterns show the same lines as those of the first class and in addition two lines corresponding to crystallographic indexes (111), first order, and (100), third order, and a strong line corresponding to a spacing of 4.10 Å. Apparently these samples contain a greater concentration of atoms at fractional distances between the corners of the cubes than the first class. Bentonites 3 and 8 and the beidellite also show a spacing of 3.30 Å., but since this spacing does not correspond to any spacing in the theoretical cube, it was probably due to some impurity in the samples.

The potash bentonite sample apparently belongs in a separate class. Its pattern contains the same lines as bentonite 7, but, in addition it contains lines corresponding to the crystallographic indexes: (112), (123), (120) second order, and (125) and (140) first order. This suggests a considerably greater distribution of atoms at fractional distances between the corners of the unit cube than in the other classes of bentonites. The structure of halloysite appears to be similar to, although less complex than, potash bentonite.

Summarizing these conclusions, we may say that the patterns of the bentonites that have been examined appear to fall into three classes of varying complexity. The simplest class has a structure apparently related to a simple cubic lattice, (i.e., a lattice with the atoms or atomic groups mostly at or near the corners of the unit cube) since the prominent lines agree with simple crystallographic indexes. Other classes have lines agreeing with more complicated indexes, involving intercepts on 1/3, 1/4, 1/5, etc. of the unit edge, and the corresponding structures, although based on a unit cube of the same size, apparently have a considerably greater distribution of their constituent atoms at fractional distances between the corners of the cube.

As has been intimated, the X-ray evidence indicates that the soil colloids possess a more complex structure and are more heterogeneous than the bentonites. Each of the soil colloid patterns contains all of the lines of at least one bentonite pattern, and some contain all of the lines of two different bentonite patterns; in addition, each contains lines foreign to the bentonite patterns but related to the unit cube; and, finally, each contains several lines not related to the unit cube. This suggests that the soil colloids may be composed of one or more bentonite or similar substances mixed with non-bentonitic material.

Because of the obvious heterogeneity of the soil colloids and the faintness and uncertainty of some of the lines, it has been found impracticable to classify the soil colloid pattern. As distinguished from bentonites, the soil colloids (with one exception) are characterized by the presence of a 1.54 Å. spacing corresponding to crystallographic index (334). Soil colloids 431 and 7083 showed the simplest diffraction patterns and are very similar to that of the potash bentonite. The pattern of soil colloid 5696 is somewhat more complex, but still it resembles that of potash bentonite (compare pl. 1, fig. 2, and pl. 2). Two of these colloids, 431 and 7083, were separated from California soils, and the third, 5696, came from Nevada; they, therefore, represent semiarid soils. The

other samples representing soils from the humid sections of America are obviously more complex structurally.

After the preceding part of this paper had been written Hendricks and Fry kindly sent us a copy of their unpublished manuscript entitled, "The results of X-ray and microscopic examination of soil colloids." This paper has subsequently been published (4). Their X-ray photographs are not strictly comparable with ours for the reason that they used a copper target, whereas we employed a molybdenum target, and also the size and types of cassettes used by them were different from ours. The calculated spacings reported in table 4, 5, 6, and 7 of their paper are in substantial agreement with those recorded in our tables 7 and 8. The montmorillonite pattern of Hendricks and Fry agrees with those of bentonite 1, 2, and 7, and the so-called Ordivician bentonite showed an exotic 3.30 spacing just as do our bentonites 3 and 8 and the potash bentonite. They suggest that the Ordivician bentonite was contaminated with quartz. As some of our materials may also have contained small quantities of quartz, it is possible to account for some of the stray lines on that basis.

In order to obtain further evidence on this point, an X-ray powder diagram was made of a sample of ground quartz. The pattern showed lines corresponding to a very, very strong 3.35 Å. spacing and to strong 4.30, 1.80, and 1.37 Å. spacings, as well as a number of weaker lines. The four strong lines might be expected to contribute to a pattern if present as an impurity, even if only in comparatively small proportion. It is interesting to note that bentonites 3 and 8, beidellite, and all of the soil colloids show evidence of quartz contamination by the presence of one or more of these lines.

We have confirmed the conclusions of Hendricks and Fry that beidellite is structurally very similar to bentonite. Their kaolinite pattern, however, is distinctly different from our bentonite patterns.

THE ZEOLITES

The term "zeolite" is often employed in discussions on the base-exchange property of soils and much use has been made by soil investigators of the synthetic preparation known as permutite or synthetic zeolite. As is well-known, permutite possesses marked base-exchange power and is undoubtedly similar in certain respects to the base-exchange material of soils. Burgess (2) holds that the calcium form of the permutite which he has synthetized is identical chemically with the natural zeolite known as scolecite.

We have investigated several natural zeolites, with the results shown in table 9. The samples used in these experiments were ground to a moderate extent only and were still definitely crystalline, as was indicated by X-ray examination. Nevertheless, practically all of the bases of stilbite were replaceable and also a very large part of those of the other zeolites. In this respect the natural zeolites differ markedly from the bentonites and the soil colloids that we have investigated. As has been pointed out already, relatively little

magnesium of the soil colloids is replaceable and much of the magnesium of bentonite cannot be replaced unless the material is ground to extreme fineness. Practically all of the bases contained in crystalline heulandite and analcite are also replaceable.

Another important difference between the natural zeolites, and bentonite and soil colloids, is found in their content of magnesium. As already shown, the bentonites are primarily magnesium compounds and the soil colloids discussed in this paper are closely related to the bentonites. This is not true, however, of the zeolites. As far as we are aware no natural magnesium zeolite has ever been reported.

X-ray studies on natural zeolites

The following is a list of the zeolites that have been examined by the X-ray method, together with the crystallographic systems in which mineralogists classify them:

Analcite	Cubic
Natrolite	Orthorhombic
Heulandite	Monoclinic
Stilbite	Monoclinic
Scolecite	Monoclinic

All of these substances gave good X-ray patterns. As might be expected from the diverse crystallographic systems to which they belong, there is a corresponding diversity in the appearances of the patterns. It is significant that none of these patterns resembles the typical patterns of the bentonites and soil colloids; consequently their base-exchange properties cannot be due to the same substances.

It might be expected that, since analcite and the bentonites belong to the same crystallographic system (cubic), the base replacement mechanism would be essentially the same in both. However, chemical studies have shown that there is a distinct difference in the behavior of these substances, complete replacement in the bentonites taking place only after prolonged grinding, whereas the bases of analcite are readily replaced with only moderate grinding treatment. The analcite structure appears to be based on a larger unit cube than the bentonites and it is possible that the difference in lattice dimensions may account for the easier replacement in analcite, since a more open structure in this substance would presumably permit a readier movement of bases within the lattice.

Several samples of synthetic permutite have also been examined with the X-ray, but, in agreement with the results of other investigators, none of these materials gave a diffraction pattern. Evidently these substances are amorphous.

Effect of heat

Numerous studies made here and elsewhere indicate that the inorganic base-exchange material of soils is a comparatively stable substance. For example the bases may be completely replaced by H ions, upon treatment with a dilute acid, without effecting decomposition of the base-exchange material to any great extent. In view of the data presented already, the fact that both the bentonites and the soil colloids are known to absorb water very readily, and that the base-exchange material of bentonite probably contains water of crystallization, a few experiments were made with reference to the effect of heat on these materials.

TABLE 9

Total bases and replaceable bases of natural zeolites

	MILLIEQUIVALENTS PER 100 GM.							
	Total bases				Replaceable bases			
	Ca	Mg	K	Na	Ca	Mg	K	Na
Natrolite	90	9	8	352	42	6	7.5	254
Stilbite	263	6	5	63	265	4	4.5	44
Scolecite	360	10	13	33	208	Trace	8.0	16

TABLE 10

Effect of heat on bentonites, soil colloids, and natural zeolites

TEMPERATURE	MILLIEQUIVALENTS OF TOTAL REPLACEABLE BASES (NH_4 ABSORBED)					
	Bentonite No. 7	Bentonite No. 3	Soil Colloid 7083	Natrolite	Stilbite	Scolecite
°C.						
Not heated	105.0	86.0	56	221.5	312.0	213.0
350	103.0	88.0	54	102.0	19.7	12.2
500	95.0	76.0	..	98.0	.....	12.5
600	52.5	19.2	19	.....	.....	.....
750	2.2	2.5	..	.....	.....	.....

The data reported in table 10 show that the soil colloids and bentonites are remarkably stable toward heat. The heating experiments were conducted in a muffle furnace controlled by a pyrometer, the samples being held in the furnace at each temperature until constancy of weight was attained. It will be noted that the temperature of 350°C. produced no effect on the replaceable bases of the soil colloid and bentonites. Above this temperature there was a gradual falling off in the content of replaceable bases, until at a temperature of 750°C. the base-exchange power was practically destroyed. On the other hand, the zeolites behaved very differently. Their power to undergo an exchange of bases with a salt solution was greatly reduced by heating to a temperature of 350°C.

It is difficult to harmonize the results of this investigation with those obtained by Steemkamp (6), who reported that the content of replaceable bases of certain soils of England is materially affected by dehydration. It is possible that the changes noted by Steemkamp were occasioned by the organic part of the exchange substance. However, we have thus far been unable to verify Steemkamp's conclusions.

A striking difference is also brought out by heating these materials to a temperature of 100°C. Both the bentonites and soil colloids lose about 10 per cent of their air-dried weight at 100°C., whereas the natural zeolites lose much less water at this temperature.

The natural zeolites are considered by mineralogists to be hydrous alumino-silicates, formed as a result of the weathering of feldspars. From a geological point of view the zeolites are relatively unstable substances. On the other hand, the clay minerals are relatively stable chemically. The colloidal material of soils is undoubtedly the most highly weathered part of the soil, representing as it does the material that is left behind after the less stable and more soluble substances have been removed by percolating water. When we consider the circumstances under which soils are formed and the conditions to which they are subjected in the natural state, it is evident that those constituents which persist in the soil must be relatively stable substances. In the event that one or more of the natural zeolites should be laid down with the soil forming materials, or be formed in the soil mass as a result of weathering processes, they would probably undergo complete decomposition under the conditions which bring about the development of a relatively mature soil profile.

GENERAL DISCUSSION

The foregoing data seem to justify the definite conclusion that the substances composing the colloidal material of the soils that have been investigated are not amorphous, as has been commonly assumed, but on the contrary that they are composed chiefly of crystalline substances. It is interesting that Hendricks and Fry (4) have found by microscopic and X-ray examination that the colloidal materials separated from many different types of soils from various parts of the United States contain definitely crystalline bodies. In agreement with our conclusions, they hold that the crystalline materials of the soil colloids examined by them belong to the clay minerals. Their results indicate that certain of the soil colloids are closely related to the montmorillonite-beidellite clays, others are similar to the potash bentonites, referred to by them as Ordivician bentonite; whereas still others are related to the halloysite type of clay minerals. Every sample which they examined was found to contain definitely crystalline material.

We have found that the replaceable bases occur not only on the surface of colloidal particles but also on the interior of the crystals. Since the X-ray analyses indicate an orderly arrangement of the atoms within the particles, and

since the exchange of bases is stoichiometric, it is safe to conclude that the base-exchange substances are true chemical compounds. The replaceable bases, therefore, are not merely adsorbed on the surface of the particles, rather the bases are an integral part of the chemical constitution of the crystals.

Ross and Shannon (5) and Wherry (7) have concluded that the crystals of colloidal bentonite are lamellar in form. Since it is only the bases that occur on or near the surface of the crystals that can be replaced, the ratio which we have found between the replaceable bases and the total base content of the unground samples of bentonite is consistent with this conclusion. The X-ray evidence as to the cubical form of the crystal lattice is also consistent with this view. It is possible that the crystalline soil colloidal material is also lamellar.

It should be pointed out, in this connection, that the double-refractive power of bentonite and various soil colloids, which has been reported by Ross and Shannon (5), Hendricks and Fry (4), and several European investigators, indicates that the crystal structure of these materials is not strictly cubical. At present, it is not possible to explain the seeming conflict of evidence on this point. It is possible that the crystal lattice is pseudo-cubic, rather than exactly cubic in form. On the other hand, certain crystalline materials, when present in the form of fine needles or thin plates, have been found to be double-refractive and at the same time to possess cubical lattice structures.

The chemical and X-ray data clearly establish the fact that the inorganic soil colloids are not zeolites. It is interesting in this connection that Gedroiz (3) has recently pointed out that the inorganic base-exchange material of soils is not zeolitic (*ungenau auch zeolithischer Teil des Bodens genannt*), on the contrary, these colloids apparently belong to the group of minerals known to mineralogists as clays. Certain of the better known clay minerals, such as kaolinite, and the less well known anauxite, are hydrogen aluminum silicates, which do not possess important base-exchange powers. On the other hand, the mortmorillonite-beidellite group of clay minerals, which characterizes the bentonites, are essentially crystalline magnesium aluminum silicates. Apparently the magnesium may be partially substituted by potassium either before crystallization takes place or subsequently, with the resulting formation of a magnesium potassium aluminum silicate. The bases that occur on or near the surface of the crystals may be replaced by calcium or sodium in the natural state, but the results of grinding experiments demonstrate that calcium and sodium do not extend into the centers of the crystals.

The Cecil colloid represents one of the oldest soil formations on the American continent. It has been derived from igneous parent material, either granite or rocks that are closely related to the granites. This colloid is characterized by a relatively low base-exchange capacity and low total base content. As pointed out in the foregoing, the crystalline material of this colloid is distinctly acidic. Hendricks and Fry (4) have investigated several samples of Cecil colloids and have concluded that they are closely related to the halloysite type of clays. Our results on a single sample of Cecil colloid are consis-

tent with this view. It is interesting to note that halloysite contains notable amounts of replaceable H ions, and the same is true of the Cecil colloid.

As is well known, the chief replaceable base of normal soils is usually calcium, This is probably because of two facts: (1) Calcium ions possess a relatively high energy of replacement, and (2) The soil solutions which come into contact with the colloidal particles contain more or less dissolved calcium derived from the weathering of various calcium minerals, including calcium carbonate. One or more of the calcium minerals are almost universally laid down with the soil-forming materials. The result is that calcium brought into solution subsequently by weathering agents, replaces more or less of the magnesium from the surface of the clay minerals. In the case of alkali soils, which contain relatively high concentrations of sodium salts, the bases that occur on or near the surfaces of the crystals are replaced in considerable part by sodium.

Soil colloid 431 represents a soil of granitic origin that was formed within a relatively recent geological period under conditions of limited rainfall. As pointed out already, the Cecil soil was also derived from granitic rock, but has been subjected to prolonged weathering under a humid climate. It is interesting that soil colloid 431 is related to the bentonites, whereas according to Hendricks and Fry the Cecil soil resembles halloysite.

It seems appropriate to state that this investigation has not been sufficiently extensive to warrant conclusions as to the nature of the base-exchange material in soils everywhere. The essential agreement between our results and those of Hendricks and Fry, based on a study of a much wider range of soil colloids, suggests, however, that the few samples which we have investigated are widely representative. We expect to expand this work so as to include a wider range of soils.

As stated already, Burgess (2) claims that the permutite which he has synthesized is identical chemically with the natural zeolite known as scolectite. If this be true, it is safe to say that the synthetic material differs materially from the base-exchange substance of soils that we have examined. As a matter of fact there is some question whether the synthetic preparation is actually identical chemically with scolecite. It seems that Burgess' conclusion was based primarily on the fact that the synthetic substance has the same percentage composition as scolecite. Such evidence, however, is not sufficient to establish chemical identity, for it is well-known, for example, that the percentage composition of the various hexose sugars is the same, yet some of these sugars are ketones whereas others are aldehydes. Among the silicates the ratio of $Al_2O_3:SiO_2$ in many different minerals is practically identical.

REFERENCES

(1) Bragg, W. H., and Bragg, W. L. 1924 X-Ray and Crystal Structure, p. 12. Harcourt, Bruce and Co., New York.

(2) Burgess, P. S. 1929 The so-called "build-up" and "break-down" of soil zeolites as influenced by reaction. *Ariz. Agr. Exp. Sta. Tech. Bul.* 28: 101–135.

(3) Gedroiz, K. K. 1929 Der adsorbierende Bodenkomplex und die adsorbierten Bodenkationen als Grundlage der genetischen Bodenklassifikation. *Kolloidchem. Beihefte.* 29: 149–260.

(4) Hendricks, S. B., and Fry, W. H. 1930 The results of X-ray and microscopic examinations of soil colloids. *Soil Sci.* 29: 457–479.

(5) Ross, C. S., and Shannon, E. V. 1926 The minerals of bentonite and related clays and their physical properties. *Jour. Amer. Ceramic Soc.* 9: 77–96.

(6) Steemkamp, J. L. 1928 The effect of dehydration of soils upon their colloid constituents. *Soil Sci.* 25: 163–182.

(7) Wherry, E. T. 1925 Bentonite as a one-dimensional colloid. *Amer. Mineralogist* 10: 120–123.

(8) Wyckoff, R. W. G. 1924 The Structure of Crystals, p. 190–195. Chemical Catalog Co., New York.

PLATE 1

X-Ray Diffraction Patterns of Bentonites and Soil Colloids

Fig 1. Interplanar spacings of different samples of bentonite.
Fig 2. The structural similarity between bentonites and soil colloids.

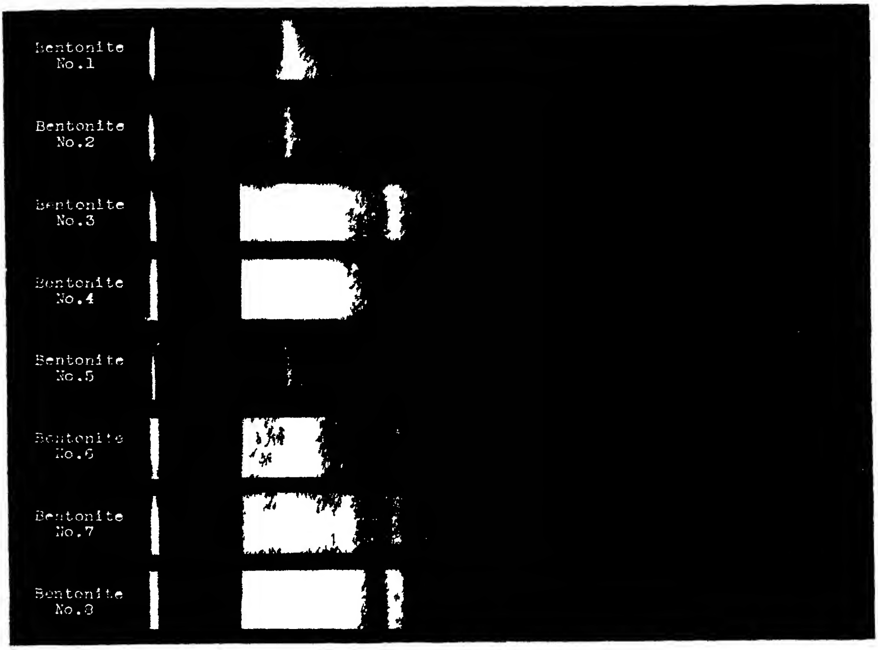

Bentonite No.1
Bentonite No.2
Bentonite No.3
Bentonite No.4
Bentonite No.5
Bentonite No.6
Bentonite No.7
Bentonite No.8

FIG. 1

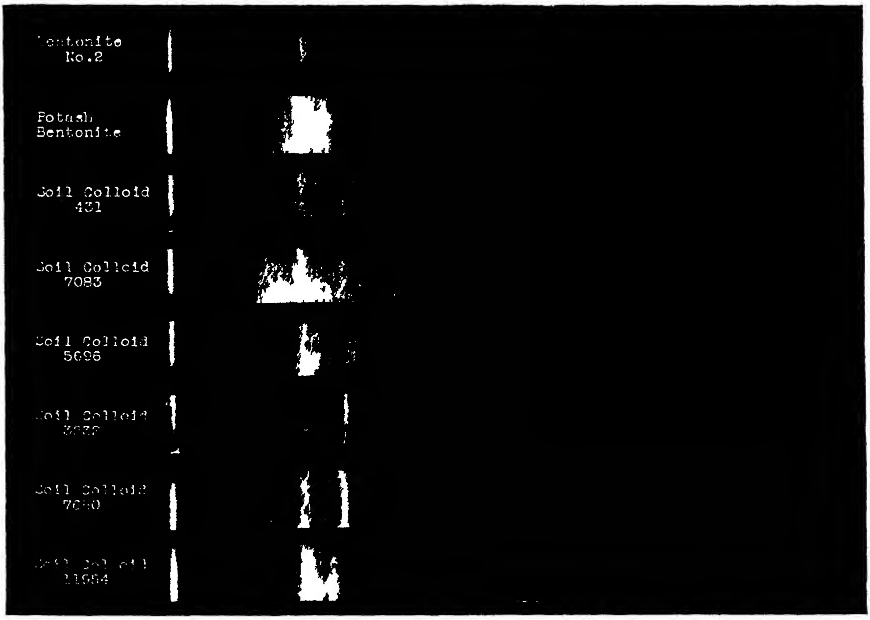

Bentonite No.2
Potash Bentonite
Soil Colloid 431
Soil Colloid 7083
Soil Colloid 5696

FIG. 2

PLATE 2

The Structural Relationship of Different Clay Minerals

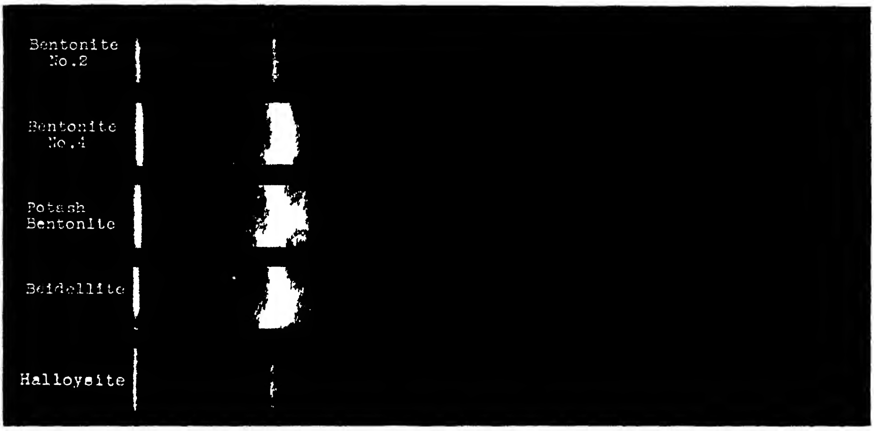

Bentonite
No.2
Bentonite
No.4
Potash
Bentonite
Beidellite
Halloysite

PLATE 3

X-RAY DIFFRACTION PATTERNS OF BENTONITE AND SOIL COLLOIDS AS AFFECTED BY GRINDING

FIG. 1. The crystalline structure of bentonites as affected by grinding.
FIG. 2 The crystalline structure of soil colloids as affected by grinding.

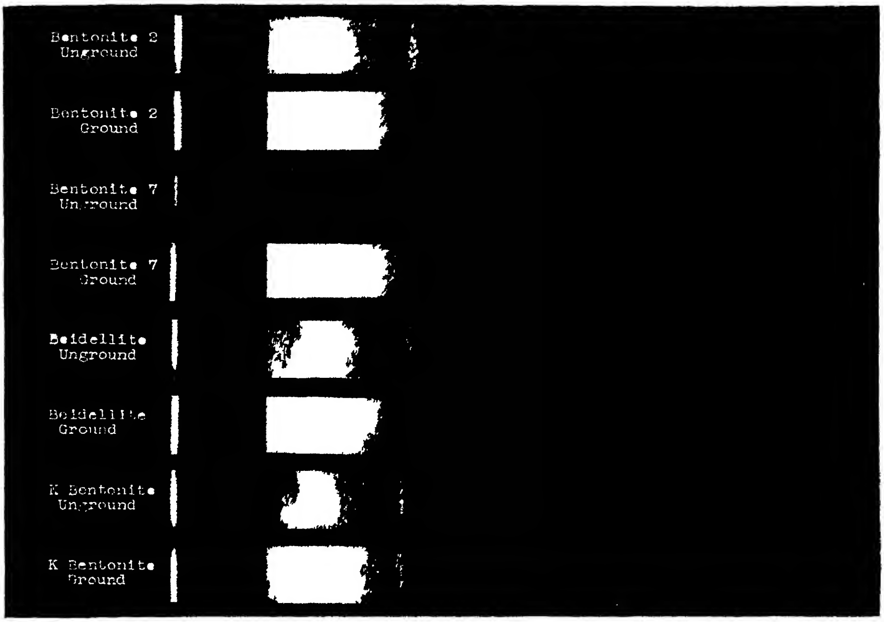

Bentonite 2
Unground
Bentonite 2
Ground
Bentonite 7
Unground
Bentonite 7
Ground
Beidellite
Unground
Beidellite
Ground
K Bentonite
Unground
K Bentonite
Ground

FIG 1

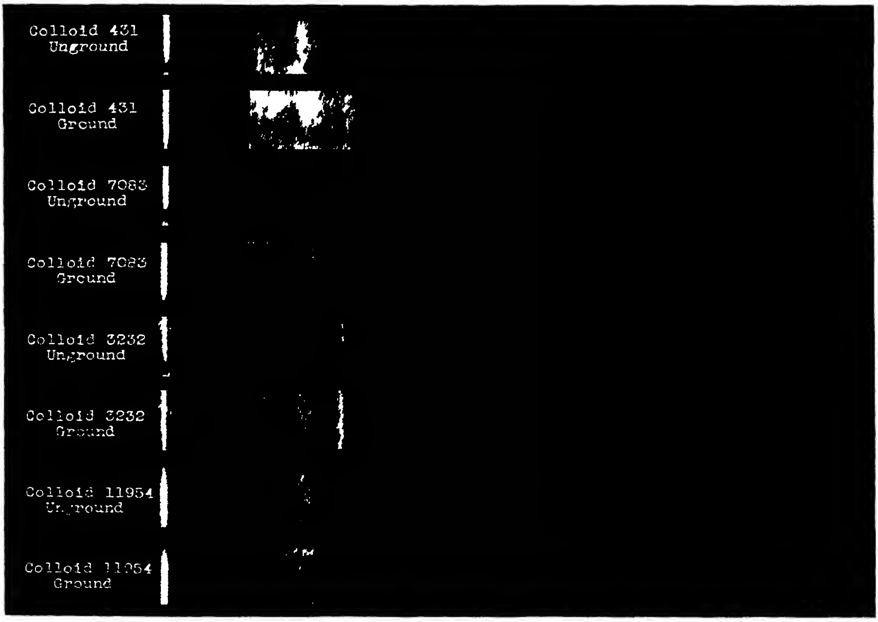

Colloid 431
Unground
Colloid 431
Ground
Colloid 7088
Unground
Colloid 7088
Ground
Colloid 3232
Unground
Colloid 3232
Ground
Colloid 11954
Unground
Colloid 11954
Ground

FIG 2

PLATE 4

X-RAY DIFFRACTION PATTERNS OF BENTONITE AND SOIL COLLOIDS AS AFFECTED BY BASE-REPLACEMENT

FIG. 1. Crystalline structure of bentonite as affected by base replacement.
FIG. 2. Crystalline structure of soil colloids as affected by base replacement.

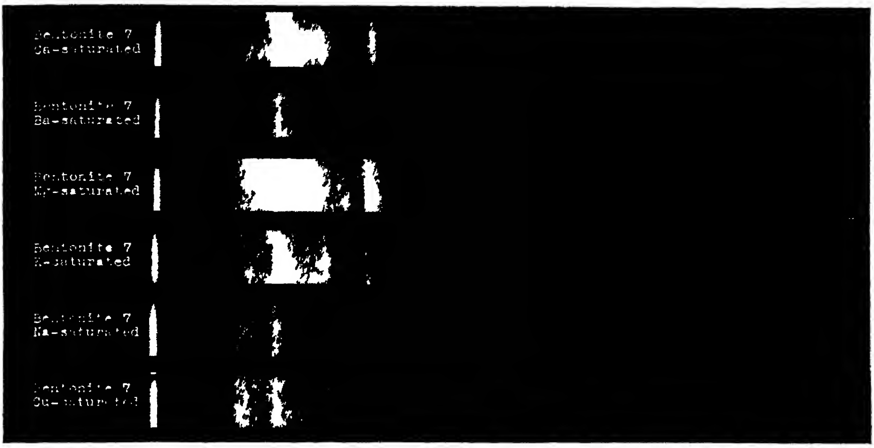

Bentonite 7
Ca-saturated
Bentonite 7
Ba-saturated
Bentonite 7
K-saturated
Bentonite 7
Na-saturated

FIG 1

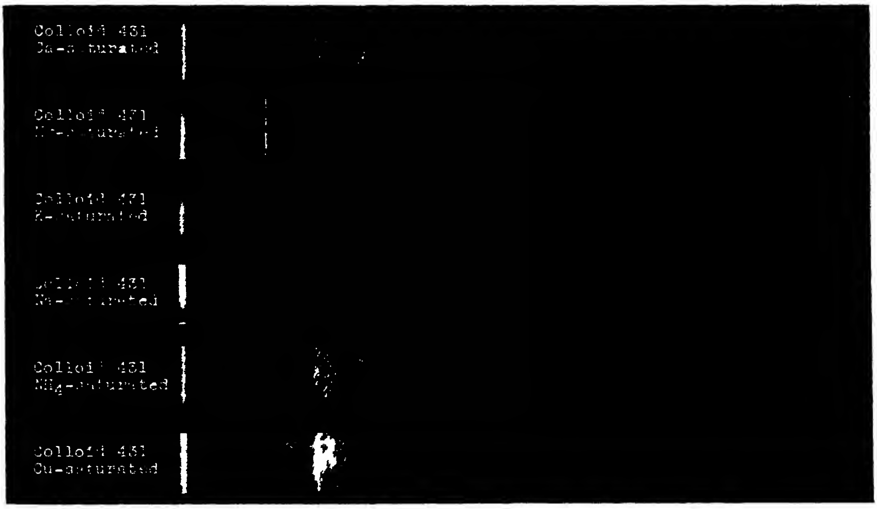
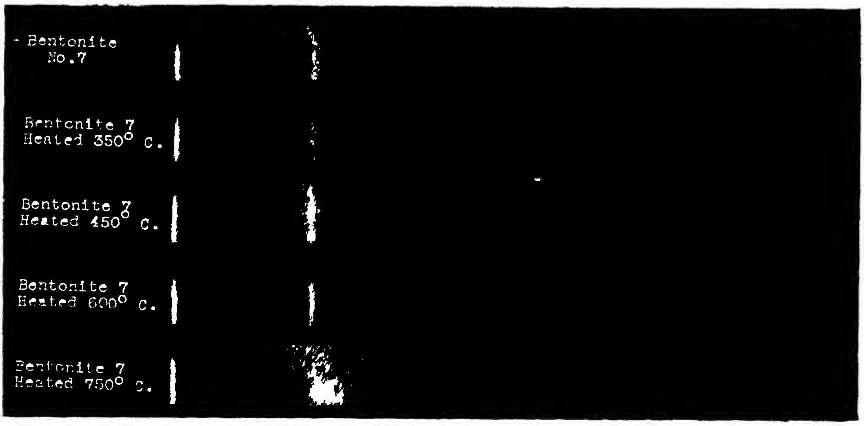

Colloid 431
Colloid 431
Colloid 431
Colloid 431
NH₄-saturated
Colloid 431
Cu-saturated

FIG 2

PLATE 5

Effect of Heat on the Crystalline Structure of Bentonite


Bentonite
No. 7
Bentonite 7
Heated 350° C.
Bentonite 7
Heated 450° C.
Bentonite 7
Heated 600° C.
Bentonite 7
Heated 750° C.

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: IV. ISOELECTRIC PRECIPITATES[1]

SANTE MATTSON

New Jersey Agricultural Experiment Station

Received for publication April 19, 1930

In the preceding section (Part III) of this series the isoelectric precipitates of aluminum and ferric "hydroxides," "phosphates," and "silicates" were discussed. In the present paper we shall deal with another class of isoelectric precipitates which are no less important for an understanding of soil processes, the so-called "humates."

The soil organic matter known as humus possesses acidic or acidoid properties, combining with bases to form "humates." These substances have been subjected to an extensive physico-chemical investigation by Odén (6), who regards them as true acids in contradistinction to the adsorption theory of Baumann and Gully (1). Whether the acid nature of humic substances is due to replaceable carboxyl hydrogen, or to hydroxyl hydrogen as in the phenols, or to hydro-carbon hydrogen as in aceto-acetic and malonic ester, or to the hydrogen of the water activated through a fixation of the hydroxyl group, is of no significance for our present study.

Humus combines with, and neutralizes bases, and resembles in this and other respects silicic, oleic, and other weak acids. It is also distinctly electronegative, migrating toward the anode in a solution of any pH. It is readily dispersible when free from electrolytes. The alkali "humates" are extremely dispersible, bordering on true solution if not actually attaining it. The "humates" of the divalent cations are not so highly dispersible. All are electronegative. The aluminum and ferric "humates," which are our present object of study, are amphoteric, and are non-dispersible at or near the iso-electric point but become highly dispersible with increasing positive or negative charge.

The neutralizing equivalent (and cation exchange capacity at the point of neutrality) of the humus acids is very high—about three times higher than that of the highly silicated mineral soil colloids such as bentonite, the equivalent of which is about 1,000 gm. (per gram equivalent base). Odén found the equivalent for "humic acid" to be about 330. For "hymatomelanic acid" (the alcohol-soluble component) he found a value of 220 by the use of various methods 7). For a sample of "humic acid" prepared according to the method of Odén, the author found an exchange capacity of 3.064 milliequivalents per gram,

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

which yields an equivalent of 326. There seems therefore to be a remarkable constancy in the neutralizing power of this complex. Where the more inactive "humin" (humuskohle) colloids were not separated from the alkali "humate" (by flocculation with NaCl) Odén found a higher equivalent. "Humic acid" from young peats shows a lower equivalent because of the presence of pectinic acids. A lower equivalent is also found whenever the "hymatomelanic acid" is not separated. For Merck's "acidum huminicum" Odén found equivalent weights between 200 and 300. "Raw humic acid" yielded values between 250 and 400 according to its origin.

Regarding the constitution of the humus complex we know nothing. Which of, and to what extent, the various organic constituents known to be present in the colloid are responsible for its acid nature is of the greatest importance for our present study. Since the relative proportions of these components vary with the nature of the original plant material, with the stage of transformation, and with the climatic and microbiologic conditions, under which the material is formed, it is obvious that there can be no constant equivalent for the humus colloid as a whole.

In the following experiments the total alkali dispersible colloid was employed. In experiments now in progress an attempt is being made to study the various components of humus from the colloid chemical standpoint. The various components of humus reported as "hemicellulose," "cellulose," and "lignin" may be very different from the corresponding materials present in the original plants in which they were synthesized. Oxidation and reduction, substitution and addition derivatives, and lactone formation would result in combinations possessing very different properties. It must be remembered that in the methods of analysis now in vogue, these delicately complex and reactive materials are subjected to such a drastic treatment that any conclusion as to their original make-up is extremely hazardous.

PREPARATION OF "Na-HUMATE"

The peat from which the humus colloid was obtained was supplied by Dr. C. E. Skinner whose description of the material reads:

> Black spruce bog, Lewis Township, Mille Lacs County, Minnesota; pH 4.0 or below, ash low (about 5 per cent), stratum of black spruce and tamarack; shrub stratum of Ledum, lowest stratum of living sphagnum. This is a true sphagnum bog, but is underlaid with remains of sedge peat. It is an uncultivated virgin bog.

Two kilos of the moist peat were digested with 1,250 cc. strong ammonia in 18 liters of distilled water, shaken, and let stand for two months. The liquid was then siphoned off from the sediment and acidified with a large excess of HCl. The precipitate was then washed on the filter until the colloid started to disperse freely. It was then placed in a large electrodialysis cell, the central compartment of which measured 10 by 13 inches and $\frac{3}{4}$ inch in thickness. The cell was put in a 110 volt D.C. circuit in series with three 100-watt lamps

placed in parallel. The current was about 2 amperes at the beginning and fell to a fraction of an ampere at the end of three days when the diffusible ions were practically all removed.

A sample of the electrodioalyzed humus, which yielded 1.12 per cent ash, was then treated on the filter with a normal solution of neutral Ca-acetate until the filtrate gave a neutral reaction, and then once with $CaCl_2$. It was then washed until the Cl-ion reaction disappeared. The Ca was now displaced with normal NH_4Cl and the Ca determined in the filtrate. The base exchange capacity was 2.857 milliequivalents per gram, which gives an equivalent weight of 350.

A "Na-humate" solution was now prepared by adding 100 cc. N NaOH to a suspension containing 35 gm. dry weight of the electrodialyzed humus and diluting to 1 liter. This makes a 0.1 N solution of Na-humate *with respect to the Na ion.*

THE ISOELECTRIC "HUMATES" OF ALUMINUM AND IRON

A series of these precipitates were prepared by mixing varying proportions of "Na-humate" with aluminum and ferric chlorides as described in the preceding section (part III: Isoelectric precipitates) in this journal. The pH was adjusted to the isoelectric point by adding NaOH to the Na-humate or HCl to the chlorides, as the case required, before the mixing of these solutions. The results are shown in tables 48 to 53b.

Starting with a mixture of three milliequivalents of $AlCl_3$ to three of Na-humate, as in table 48, we see that the system remains electronegative over the entire range of pH values, which covers the zone of most rapid flocculation. If we assumed that each equivalent of "humate" displaces quantitatively one equivalent of Cl, we would expect the complex with the aforementioned proportions to remain electronegative because the humate ion would probably not be dissociated by the complex. The humate systems differ from the silicate and phosphate systems in that the humus, which is colloidal, is completely precipitated with the aluminum and ferric "humate" complex, leaving, in all cases, a colorless solution. But it is very doubtful whether all the humus combines with the metal ions. We are probably dealing with an equilibrium reaction similar to the reactions with the phosphate and silicate. Since the free humus is carried down with the precipitate there is no direct way of telling to what extent combination takes place, but, as we shall see later, this information comes in an indirect and very interesting way (section V).

The OH ions undoubtedy compete with the humate as with the phosphate and silicate ions and the diffusible Cl ions probably persist in the complex up to fairly high pH values. The fact that the complex in table 48 remains negative must be due to the condition that the anionic dissociation of the Cl ions is more than balanced by the cationic dissociation of the free humus complex.

Passing now to a proportion of six milliequivalents of Al to three of the humate, as in table 49, we note that the complex becomes isoelectric between a pH

5.0 and 4.2, is weakly positive at 4.2 and returns to the isoelectric conditions again at a pH of 3.8. At still lower pH values it remains weakly negative. We

TABLE 48

The $AlCl_3$ + Na-humate, system no. 51

A. 1.0 millimol $AlCl_3$ in 1,000 cc.
B. 3.0 millimols Na-humate in 1,000 cc.

SOLUTION A, 20 cc. + HCl MILLIMOLS*	SOLUTION B	FLOCCULATION:		μ/sec 1 volt/cm.	pH
		After mixing	Overnight		
	cc.				
0.0	20	Slow	xx	......	...
0.005	20	Instant	xxxx	−1.26	4.5
0.01	20	Instant	xxxx	−1.00	4.2
0.03	20	Instant	xxxx	−0.53	3.7
0.05	20	Instant	xxxx	......	...
0.10	20	Rapid	xxxx	−0.49	...
0.15	20	Rapid	xxxx	......	...
0.30	20	Rapid	xxxx	−0.23	...

* Plus enough water to make a total volume of 50 cc.

TABLE 49

The $AlCl_3$ + Na-humate, system no. 52

A. 2.0 millimols $AlCl_3$ in 1,000 cc.
B. 3.0 millimols Na-humate in 1,000 cc.

SOLUTION A, 20 cc. + HCl MILLIMOLS	SOLUTION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION:		μ/sec. 1 volt/cm	pH
		After mixing	Overnight		
0.0	0.075	0	0	......	...
0.0	0.065	0	x	......	...
0.0	0.060	Rapid	xxx	−1.95	...
0.0	0.050	Instant	xxxx	−0.38	5.0
0.0	0.025	Instant	xxxx	+0.18	4.2
0.0	0.0	Instant	xxxx	±0.0	3.8
0.05	0.0	Rapid	xxxx	−0.15	...
0.15	0.0	Rapid	xxxx	......	...
0.30	0.0	Rapid	xxxx	−0.23	...

Isoelectric mixture:
2.0 millimols $AlCl_3$ in 1,000 cc.
3.0 millimols Na-humate + 1.65 millimols NaOH in 1,500 cc.
pH 4.5.
Al_2O_3 in solution: 0.195 millimol.
Humus in solution: None.

Composition of floc: $\frac{1.05 \text{ gm. humus}}{0.805 \text{ millimol } Al_2O_3} = 1.305$ gm. humus per millimols Al_2O_3.

have here hit upon a proportion in which the amphoteric character of the complex is just developing. There are here two isoelectric points. There is

just a slight dip across the isoelectric line and then a return to the negative condition, as it is shown in figure 8.

This return to the negative condition was not observed in the aluminum and ferric "phosphates" and "silicates." The reason for this difference in behavior is to be sought in the colloidal nature of the humus complex itself. When the pH in the phosphate and silicate system is lowered these complexes become increasingly electropositive because the diffusible Cl ions, highly dissociated by the complex, enter in ever-increasing numbers until the complex undergoes a complete molecular and ionic dispersion. Neither the anions nor the cations remain colloid-dispersed [except in concentrated silicate systems where

TABLE 50

The $AlCl_3$ + Na-humate, system no. 53

A. 4.0 millimols $AlCl_3$ in 1,000 cc.
B. 3.0 millimols Na-humate in 1,000 cc.

SOLUTION A	SOLUTION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION:		μ/sec. 1 volt/cm.	pH
		After mixing	Overnight		
cc.					
20	0.19	0	x	......	...
20	0.18	Rapid	xxxx	−1.38	5.8
20	0.16	Instant	xxxx	+0.84	4.75
20	0.14	Instant	xxxx	+1.18	4.5
20	0.12	Instant	xxxx	+1.18	4.4
20	0.10	Instant	xxxx	+1.02	4.3
20	0.05	Instant	xxxx	+0.80	4.2
20	0.0	Instant	xxxx	+0.34	3.8

Isoelectric mixture:
4.0 millimols $AlCl_3$ in 1,000 cc.
3.0 millimols Na-humate + 8.38 millimols NaOH in 1,500 cc.
pH 5.15.
Al_2O_3 in solution: 0.029 millimol
Humus in solution: None.

Composition of floc: $\frac{1.05 \text{ gm. humus}}{1.971 \text{ millimols } Al_2O_3}$ = 0.533 gm. humus per millimols Al_2O_3.

a decrease in the positive or an increase in the negative charge of the remaining complex may be observed at low pH values (5)].

In the humate systems, in which the negative component is almost wholly colloidal, the effect of a lowering of the pH will be in two directions. The amphoteric sesquioxide component will become more strongly electropositive as the number of Cl ions in combination increases and the positive charge of the complex will therefore increase at first. But as the solubility of this component increases with the chloridation it becomes quantitatively weaker and the complex as a whole will grow less positive or more negative because the negative component (humus) remains colloid-dispersed in the complex.

TABLE 51A

The $AlCl_3$ + Na-humate, system no. 54

A. 8.0 millimols $AlCl_3$ in 1,000 cc.
B. 3.0 millimols Na-humate in 1,000 cc.

SOLUTION A	SOLUTION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION:		μ/sec. 1 volt/cm.	pH
		After mixing	Overnight		
cc.					
20	0.44	0	x	......	...
20	0.42	Rapid	xxx	−1.90	...
20	0.40	Instant	xxxx	−0.98	6.1
20	0.38	Instant	xxxx	+1.44	4.8
20	0.36	Instant	xxxx	+2.16	4.5
20	0.34	Instant	xxx	+2.16	4.4
20	0.32	Instant	xxx	+2.02	4.35
20	0.20	Instant	xxxx	+1.90	4.3
20	0.0	Instant	xxxx	+0.67	3.8

Isoelectric mixture:
8.0 millimols $AlCl_3$ in 1,000 cc.
3.0 millimols Na humate + 19.6 millimols NaOH in 1,500 cc.
pH 5.5.
Al_2O_3 in solution: 0.039 milimols.
Humus in solution: None.

Composition of floc: $\frac{\text{1.05 gm. humus}}{\text{3.961 millimol } Al_2O_3}$ 0.265 gm. humus per millimol Al_2O_3.

TABLE 51B

The $FeCl_3$ + Na-humate, system no. 55

A. 8.0 millimols $FeCl_3$ in 1,000 cc.
B. 3.0 millimols Na-humate in 1,000 cc.

SOLUTION A	SOLUTION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION:		μ/sec. 1 volt/cm.	pH
		After mixing	Overnight		
cc.					
20	0.44	0	x	......	...
20	0.42	Rapid	xxx	−2.33	6.0
20	0.40	Instant	xxxx	−1.00	5.1
20	0.38	Instant	xxxx	+0.87	4.0
20	0.36	Instant	xxxx	......	3.5
20	0.34	Instant	xxxx	+2.02	3.3
20	0.30	Rapid	xxxx	......	...
20	0.20	Slow	xxxx	+1.89	...
20	0.10	Slow	xxxx	......	...
20	0.0	Rapid	xxx	+1.60	...

Isoelectric mixture:
8.0 millimols $FeCl_3$ in 1,000 cc.
3.0 millimols Na-humate + 19.46 millimols NaOH in 1,500 cc.
pH 4.55.
Fe_2O_3 in solution: 0.212 millimol.
Humus in solution: None.

Composition of floc: $\frac{\text{1.05 gm. humus}}{\text{3.788 millimols } Fe_2O_3}$ 0.277 gm. humus per millimol Fe_2O_3.

The two isoelectric points in table 49 differ electrokinetically as follows: At the first point, pH 4.5, the electropositive component is quantitatively greater but qualitatively weaker than at the second point, pH 3.8. The total number of positive charges is not necessarily the same, because the cationic dissociation of the humus complex undoubtedly varies with the pH. It is generally maintained that the tri-valent cations are responsible for the positive charge. If this were so then the positive charge should increase as these cations become more numerous with a lowering of the pH.

TABLE 52A

The $AlCl_3$ + Na-humate, system no. 56

A. 10.0 millimols $AlCl_3$ in 1,000 cc.
B. 2.0 millimols Na-humate in 1,000 cc.

SOLUTION A	SOLUTION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION: After mixing	FLOCCULATION: Overnight	μ/sec. 1 volt/cm.	pH
cc.					
20	0.58	0	x	......	...
20	0.56	Slow	xxx	−2.02	7.0
20	0.54	Instant	xxxx	−0.76	6.6
20	0.52	Instant	xxxx	+1.78	5.5
20	0.50	Instant	xxx	+1.90	...
20	0.48	Rapid	xx	+2.00	...
20	0.46	0	x	......	...
20	0.44	0	x	......	...
20	0.40	0	x	......	...
20	0.30	0	x	......	...
20	0.20	Rapid	xx	+2.16	...
20	0.10	Instant	xxx	+1.84	4.1
20	0.0	Instant	xxxx	+0.76	3.7

Isoelectric mixture:
10.0 millimols $AlCl_3$ in 1,000 cc.
2.0 millimols Na-humate + 26.7 millimols NaOH in 1,500 cc.
pH 6.2.
Al_2O_3 in solution: None.
Humus in solution: None.

Composition of floc: $\frac{\text{0.70 gm. humus}}{\text{5.0 millimols } Al_2O_3}$: 0.140 gm. humus per millimol Al_2O_3.

The analysis represents the first isoelectric point. The composition of the floc is expressed in the number of grams of humus per millimol sesquioxide. To express the humus in milliequivalents would be meaningless, for even if we accept the base exchange value as the equivalent, the ratios would be misleading since the humus is quantitatively precipitated whether in combination or not.

In table 50 we have only one isoelectric point within the range covered. But as noted by the migration velocities and by the corresponding curve in

figure 8 a displacement toward a seond isoelectric point is evident. The greater proportion of Al expresses itself in a higher isoelectric pH (5.15) and in a stronger positive charge at lower pH values. There is, however, as yet no tendency to form a stable positive sol.

In table 51a with a proportion of 24 milliequivalents of $AlCl_3$ to 3 of Na-humate the isoelectric pH has climbed to 5.5. On the positive side there is a high maximum charge and then a gradual decline. There is here some tendency to stability as indicated by *XXX* on the positive side.

TABLE 52B

The $FeCl_3$ + Na-humate, system no. 57

A. 10.0 millimols $FeCl_3$ in 1,000 cc.
B. 2.0 millimols Na-humate in 1,000 cc.

SOLUTION A	SOLUTION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION: After mixing	FLOCCULATION: Overnight	μ/sec. 1 volt/cm.	pH
cc.					
20	0.58	Slow	x	......	...
20	0.56	Instant	xxxx	−2.52	6.5
20	0.54	Instant	xxxx	±0.0	5.4
20	0.52	Instant	xxxx	+1.26	4.0
20	0.50	Instant	xxxx	+1.68	3.6
20	0.48	Rapid	xxxx	+2.02	3.3
20	0.46	Slow	xxxx	+2.16	3.2
20	0.40	0	xxxx	+2.16	...
20	0.30	0	xxxx	+1.90	...
20	0.20	0	xxxx	+1.90	...
20	0.10	0	xxxx	+1.68	...
20	0.0	Slow	xxx	+1.60	...

Isoelectric mixture:
10.0 millimols $FeCl_3$ in 1,000 cc.
2.0 millimols Na-humate + 27.0 millimols NaOH in 1,500 cc.
pH 5.4.
Fe_2O_3 in solution: 0.091 millimol.
Humus in solution: None.

$$\text{Composition of floc: } \frac{0.70 \text{ gm. humus}}{4.909 \text{ millimols } Fe_2O_3} = 0.141 \text{ gm. humus per millimol } Fe_2O_3.$$

In table 51b the porportion of Fe is the same as the proportion of Al in 51a. The same holds for tables 52a and b, and 53a and b. We meet here the same differences in the isoelectric pH values as in the chloridated and sulfated "hydroxides," the "phosphates," and the "silicates" (except the most highly silicated ones). The isoelectric pH of the ferric "humates" is considerably lower than that of the Al-"humates." The comparison can best be shown in figure 8. Table 51b shows some tendency toward stability at very low pH values on the positive side, as seen in the "after mixing" column. The next day the flocculation was, however, "regular," in a single zone. Such differences are due to a "drift" in the charge and in the isoelectric point on standing.

In tables 52a and b, where the proportion is 30 milliequivalents chloride to 2 of humate, the isoelectric pH has increased to 6.2 in the aluminum and to 5.4 in the ferric system. The positive charge reaches a high peak and the aluminum "humate" complex becomes quite stable between the pH values 5.5 and 4.1, giving rise to two zones of flocculation, the so-called "irregular series." After being mixed, the ferric "humate" shows stability below a pH of 3.2, but this is only a transient effect, for the following day the flocculation was continuous.

In tables 53a and b, with a ratio of 30 milliequivalents chloride to 1 of humate, the isoelectric pH is 6.8 for the aluminum and 5.95 for the ferric

TABLE 53A

The $AlCl_3$ + Na-humate, system no. 58

A. 10.0 millimols $AlCl_3$ in 1,000 cc.
B. 1.0 millimol Na-humate in 1,000 cc.

SOLUTION A	SOLUTION B, 20 CC. + NaOH MILLIMOLS	FLOCCULATION: After mixing	FLOCCULATION: Overnight	μ/sec. 1 volt/cm.	pH
cc.					
20	0.60	Slow	xxx	−2.00	8.0
20	0.59	Rapid	xxxx	−1.78	7.8
20	0.58	Instant	xxxx	−1.58	7.5
20	0.57	Instant	xxxx	−1.16	7.0
20	0.56	Instant	xxxx	+0.20	6.7
20	0.54	Slow	xxxx	+1.78	5.8
20	0.52	0	x	......	...
20	0.45	0	0	......	...
20	0.30	0	0	......	...
20	0.15	0	0	......	...
20	0.0	Rapid	xxxx	+0.88	3.7

Isoelectric mixture:
10.0 millimols $AlCl_3$ in 1,000 cc.
1.00 millimol Na-humate + 28.07 millimols NaOH in 1,500 cc.
pH 6.8.
Al_2O_3 in solution: None.
Humus in solution: None

Composition of floc: $\frac{0.35 \text{ gm. humus}}{5.0 \text{ millimols } Al_2O_3}$ = 0.07 gm. humus per millimol Al_2O_3.

"humate." The stable zones on the positive side have now become permanent. It is of interest to note that where the stability of the aluminum humate ends, i.e., at pH 3.7, the stable condition of the ferric "humate" just commences. Such differences in behavior, if properly interpreted, must express themselves in the formation of the podzol horizons.

Figure 8 brings out an interesting relationship between the electropositive maxima and the pH. In the case of the aluminum "humates" these maxima coincide with a pH of 4.4 to 4.5 whereas the ferric "humates" attain a maximum positive charge at a pH between 3.2 and a slightly lower value. This

means that the amphoteric sesquioxide constituents attain, as colloids, a maximum electropositive activity at these points, i.e., a maximum electrical neutralizing power (5). Beyond these points, at still lower pH values, the sesquioxide constituent combines with more and more HCl and become undoubtedly more strongly electropositive, but true solution takes place here so rapidly that the total "electropositive quantity" in combination with the negative humus actually decreases, with the result that the complex as a whole becomes less positive.

The pH of the maximum electropositive activity of the sesquioxides will, of course, depend upon the nature of the diffusible anion. The aforementioned

TABLE 53B

The $FeCl_3$ + Na-humate, system no. 59

A. 10.0 millimols $FeCl_3$ in 1,000 cc

B. 1.0 millimol Na-humate in 1,000 cc.

SOLUTION A, 20 cc. + HCl MILLIMOLS	SOLUTION B, 20 cc + NaOH MILLIMOLS	FLOCCULATION:		μ/sec. 1 volt/cm.	pH
		After mixing	Overnight		
0.0	0.60	Slow	xx	......	...
0.0	0.58	Instant	xxxx	−1.78	6.7
0.0	0.57	Instant	xxxx	−0.92	6.2
0.0	0.56	Instant	xxxx	+1.00	5.7
0.0	0.54	Rapid	xxxx	......	4.1
0.0	0.52	Slow	xxxx	+2.02	3.7
0.0	0.30	0	0	......	...
0.0	0.0	0	0	......	...
0.04	0.0	0	0	......	...
0.10	0.0	Slow	xxx	+1.26	...
0.40	0.0	Rapid	xxxx	+1.08	...

Isoelectric mixture:
10.0 millimols $FeCl_3$ in 1,000 cc.
1.0 millimol Na-humate + 28.25 millimols NaOH in 1,500 cc.
pH 5.95.
Fe_2O_3 in solution: 0.025.
Humus in solution: None.

Composition of floc: $\frac{0.35 \text{ gm. humus}}{4.975 \text{ millimols } Fe_2O_3} = 0.0704$ gm. humus per millimol Fe_2O_3.

results apply only to the chlorides. The ferric chloride, being more hydrolyzed, requires a higher acidity both for the electrical neutralization and for the positive maximum of the humate complex.

The natural soil colloid complex being the product of the interaction between a number of different ions it seemed of interest to study the electrokinetic behavior and the composition of the floc of a few, variously composed systems.

Tables 54 and 55 show how the phosphate and the humate ions and the silicate and humate ions compete in combining with the aluminum ion. We must however not lose sight of the OH and the Cl anions, since these are both present and both compete with the others for a place in the complex. Whereas in the preceding systems we were dealing with three different anions we shall here introduce four different anions, counting the humus complex as a single anion.

In order to make the study as comparative as possible, the proportion of $AlCl_3$ to Na_2HPO_4 and to Na_2SiO_3 was taken as in tables 35 and 38a, respectively. In making any comparison it must, however, be remembered that the introduction of the humate modified the pH of the zone of flocculation as well as the isoelectric pH. The latter was, of course, lowered because of the addition of an extra, strongly electronegative component, the humus. The two milliequivalents of Na-humate lowered the isoelectric pH in the phosphated system from 5.6 to 3.9 and in the silicated system from 6.25 to 5.5.

TABLE 54

The Al-"phospho-humate," system no. 60

A. 5.0 millimols $AlCl_3$ in 1,000 cc.

B. 5.482 millimols Na_2HPO_4 + 2.0 millimols Na-humate } in 1,000 cc.

SOLUTION A, 20 cc. + HCl MILLIMOLS	SOLUTION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION: After mixing	FLOCCULATION: Overnight	μ/sec. 1 volt/cm.	pH
0.0	0.10	0	x	......	...
0.0	0.08	Slow	xxx	−1.84	...
0.0	0.06	Instant	xxxx	−0.69	4.4
0.0	0.04	Instant	xxxx	+0.13	3.8
0.0	0.02	Instant	xxxx	+0.48	3.5
0.0	0.0	Rapid	xxxx	+0.41	3.4
0.10	0.0	Rapid	xxxx	+0.18	3.1
0.30	0.0	Rapid	xxxx	+ slight	...
0.50	0.0	Rapid	xxxx	− slight	...

Isoelectric mixture:

5.0 millimols $AlCl_3$ in 1,000 cc.

5.482 millimols Na_2HPO_4 | 2.0 millimols Na-humate | 2.15 millimols NaOH } in 1,500 cc.

pH 3.9.

PO_4 in solution: 1.838 millimols.

Al_2O_3 in solution: 0.088 millimol.

Composition of floc: $Al_2O_3\,(P_2O_5)_{0.743}$

In absence of humus (pH 5.6): $Al_2O_3\,(P_2O_5)_{0.769}$ [see table 35, part III].

The electronegative effect of the humate is further seen in a recurrence of a maximum in positive charge between pH 3.8 and 3.4 in the phosphated system and between 4.5 and 4.2 in the silicated system. At still lower pH values the phosphated system again becomes isoelectric.

The composition of the isoelectric floc is interesting, for it shows, as might be predicted, that the ions displace one another. The displacement of the PO_4 ion by the "humate" is not very great but it is greater than it appears to be because at a pH of 3.9 the aluminum would, in the absence of the "humate," have combined with considerably more PO_4 than was found in combination in the precipitate, isoelectric at pH 5.6. This is made evident by an extrapolation of the aluminum "phosphate" curve in figure 10. It must be remembered also that there was only 2 milliequivalents of "humate" to 15 of $AlCl_3$

The SiO_3 ion, on the other hand, is strongly displaced by the "humate." From the aluminum "silicate" curve in figure 10, we see that, at a pH of 5.5

TABLE 55

The Al-"silico-humate," system no. 61

A. 5.0 millimols $AlCl_3$ in 1,000 cc.
B. 6.713 millimols Na_2SiO_3 + 2.0 millimols Na-humate in 1000 cc.

SOLUTION A, 20 cc + HCl MILLIMOLS	SOLUTION B	FLOCCULATION:		μ/sec 1 volt/cm.	pH
		After mixing	Overnight		
	cc.				
0.0	20	0		......	...
0.02	20	Slow	xxx	−1.80	...
0.03	20	Instant	xxxx	−1.38	6.3
0.04	20	Instant	xxxx	−0.56	5.9
0.05	20	Instant	xxxx	+1.00	4.9
0.06	20	Instant	xxxx	+1.26	4.5
0.08	20	Instant	xxxx	+1.60	4.3
0.10	20	Rapid	xxxx	+1.32	4.2
0.20	20	Rapid	xxxx	+1.26	4.1

Isoelectric mixture:
5.0 millimols $AlCl_3$ + 2.18 millimols HCl in 1,000 cc.
6.713 millimols Na_2SiO_3 + 2.0 millimols Na-humate in 1,500 cc.
pH 5.5.
SiO_2 in solution: 4.636 millimols.
Al_2O_3 in solution: trace.
Composition of floc: $Al_2O_3\ (SiO_2)_{0.83}$.
In absence of humus (pH 6.25): $Al_2O_3\ (SiO_2)_{1.63}$ [see table 38a, part III].

there should be about 2.20 mols SiO_2 per mol Al_2O_3 in the isoelectric precipitate. The addition of two millimols of Na-"humate" has reduced the SiO_2 content in the isoelectric precipitate to 0.83 mols per mol Al_2O_3.

This fact is of the greatest significance in relation to the de-silication of the soil complex in the process of podzol formation. That this displacement of SiO_2 by humus takes place in nature is shown by the work of Tamm (7). His figures would, however, have been more valuable from this point of view if the analyses had represented the colloidal fraction separately. The author would like to emphasize the importance and necessity of determining the composition

of the colloidal fraction of soils as represented by the products of hydrolytic decomposition, i.e., the synthetic colloidal complex as distinguished from crystalline fragments (rock flour). For this purpose it would be desirable to work with fractions below 0.2μ in diameter. This is deemed absolutely necessary if we are going to learn anything about the formation and degradation of this complex under natural conditions and about the development of soil horizons. The complete analysis of a soil is of little or no value in a study of this kind. Saturated with sodium most soil colloids will readily disperse to dimensions below 0.2μ and can be separated with the supercentrifuge.

ISOELECTRIC ALUMINUM AND FERRIC "PHOSPHO-SILICATES"

Tables 56a and b and 57a and b show the influence of the PO_4 and SiO_3 ions when present in the same system with $AlCl_3$ and $FeCl_3$.

Since these experiments were not carried out with the same proportions of phosphate and silicate as in the single systems no direct comparison can be made. But the quantities of $AlCl_3$ and $FeCl_3$ were kept at 5 millimols as before. We may therefore compare the composition of these precipitates with the composition—concentration curves in figure 6 (part III) and with the composition—pH curves in figure 10, which represent the single "phosphates" and "silicates."

The comparison brings out that the PO_4 ion causes a very extensive displacement of the silicate ion. We should therefore expect the soil colloidal complex formed from phosphatic rocks to possess a high PO_4 content and to be correspondingly low in SiO_2. A more or less highly phosphated and desilicated complex must also result when phosphatic solutions, as in the leachings from guano beds, react with the products of rock weathering. The PO_4 ion must enter to some extent in all soil colloids since both ground water and sea water contain this ion in small but, if the displacing power is considered, appreciable quantities.

Clarke (2) gives a table of analysis of phosphatic deposits from different parts of the world. The average percentage composition of six samples is as follows: P_2O_5, 39.0; SiO_2, 4.2; Al_2O_3, 20.5; and Fe_2O_3, 6.6. The other constituents are here ignored. In mols P_2O_5 and SiO_2 per mol $Al_2O_3 + Fe_2O_3$ we find the following average composition:

$$R_2O_3 \cdot (P_2O_5)_{1.14} \cdot (SiO_2)_{0.29}$$

which is something of the same order of combination as the aforementioned isoelectric "phospho-silicates."

It would obviously be absurd to express materials of such complex composition in the form of a simple stoichiometric formula, as the mineralogist often delights in doing. When this fails the material is declared "impure." The author would like to ask which part of the complex is to be considered as impurity? From the foregoing it is quite evident that there are any number of

silicated, phosphated, hydroxylated, humated, sulfated,—complexes; a different one in each sample of soil.[2]

Returning to a discussion of the tables we note by a comparison with the curves in figure 6 and 10 that the SiO_3 ion does not displace the PO_4 ion to any apparent degree. In fact if we plot the phosphate content in the floc, and the end concentration as in figure 6, we find that the presence of the silicate has caused more of the PO_4 ions to combine. The same phenomenon was observed by Ghosh (3) who found that a mixture of alumina and silica gel adsorbed a maximum amount of PO_4 from $Ca(H_2PO_4)_2$ when the gel contained 30 per cent SiO_2. This anomaly will be reserved for further discussion in connection with

TABLE 56A

The aluminum "phospho-silicate," system no. 17

A. 5.0 millimols $AlCl_3$ in 1,000 cc.
B. 15.0 millimols Na_2SiO_3 + 10 millimols H_3PO_4 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION:		μ/sec. 1 volt/cm.	pH
		After mixing	Overnight		
cc.	*cc.*				
20	9.0	Slow	xxxx	......	4.4
20	9.2	Instant	xxxx	+0.89	5.2
20	9.4	Instant	xxxx	+0.23	5.5
20	9.6	Instant	xxxx	−0.36	5.7
20	10.0	Instant	xxxx	−0.92	5.9
20	11.0	Opal	xxxx	......	...

Isoelectric mixture:
5.0 millimols $AlCl_3$ in 1,000 cc.
7.125 millimols Na_2SiO_3 + 4.75 millimols H_3PO_4 in 1,500 cc.
pH 5.6.
SiO_2 in solution: 6.796 millimols.
PO_4 in solution: 0.941 millimol.
Composition of floc: $Al_2O_3\ (SiO_2)_{0.132}\ (P_2O_5)_{0.762}$.

the adsorption of anions and cations by the various isoelectric precipitates at various H-ion concentrations (section VI).

A GRAPHIC REVIEW OF THE ISOELECTRIC PRECIPITATES

Figures 8, 9, and 10 give a graphic representation of the relationship between the electrokinetic behavior, the pH, and the isoelectric composition.

Figure 8 shows the direction and speed of electrical migration of aluminum

[2] In view of this fact the recent announcement of "the origin, nature and isolation of the inorganic base exchange compound in soil" (8) loses in significance, although the announcement has been widely heralded in the press as a discovery of the greatest importance. Any criticism would, however, be premature because thus far only the title of this paper has been published in a scientific journal.

and ferric "humates" at various H-ion concentrations. The figure on each curve indicates the number of grams of humus precipitated with each millimol sesquioxide at the isoelectric point.

Figure 9a shows the electrokinetic behavior at various pH values of: (*a*) aluminum "hydroxide" precipitated from the chloride; isoelectric composition: 0.0048 milliequivalent Cl per millimol Al_2O_3, (*b*) aluminum "hydroxide" from the sulfate; isoelectric composition: 0.0737 millimol SO_4 per millimol Al_2O_3, and (*c*) three aluminum "silicates," isoelectric composition as indicated.

Figure 9b: (*a*) ferric "hydroxide" from ferric chloride; isoelectric composition: 0.0052 milliequivalent Cl per millimol Fe_2O_3, and (*b*) three ferric "silicates;" isoelectric composition as indicated.

TABLE 56B

The ferric "phospho-silicate," system no. 18

A. 5.0 millimols $FeCl_3$ in 1,000 cc.

B. 15.0 millimols Na_2SiO_3 + 10 millimols H_3PO_4 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION: After mixing	FLOCCULATION: Overnight	μ/sec. 1 volt/cm.	pH
cc.	*cc.*				
20	8.2	Opal	0	....	...
20	8.4	Slow	xxxx	+1.44	3.8
20	8.6	Instant	xxxx	+1.21	4.0
20	8.8	Instant	xxxx	+0.64	4.2
20	9.0	Instant	xxxx	− slight	4.6
20	9.2	Instant	xxxx	−0.82	5.0
20	9.4	Rapid	xxxx	....	...
20	10.0	Opal	0	....	...

Isoelectric mixture:
5.0 millimols $FeCl_3$ in 1,000 cc.
6.75 millimols Na_2SiO_3 + 4.5 millimols H_3PO_4 in 1,500 cc.
pH 4.2.
SiO_2 in solution: 6.535 millimols.
PO_4 in solution: 0.299 millimol.
Composition of floc: $Fe_2O_3(SiO_2)_{0.087}(P_2O_5)_{0.840}$.

Figure 9c shows the relation between the pH and the charge of three aluminum "phosphates" and one ferric "phosphate" isoelectric composition as indicated. The corresponding tabulations will be found in part III.

Figure 10 shows the relation between the pH and the isoelectric composition of the different precipitates.

THE SESQUIOXIDE-SOIL COLLOID SYSTEMS

The various soil colloids can be isoelectrically flocculated together with the sesquioxides in exactly the same manner as the humus colloid. The reaction of the $AlCl_3$ and $FeCl_3$ with the mineral soil colloids, however, differs quantita-

TABLE 57A

The aluminum "phospho-silicate," system no. 21

A. 5.0 millimols $AlCl_3$ in 1,000 cc.

B. 7.5 millimols Na_2SiO_3 } 5.114 millimols Na_2HPO_4 } 15.0 millimols NaOH } in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION:		μ/sec 1 volt/cm.	pH
		After mixing	Overnight		
cc.	*cc.*				
20	6.9	Opal	0	......	...
20	6.95	Opal	xx	......	...
20	7.0	Instant	xxxx	+0.82	6.4
20	7.1	Instant	xxxx	±0.0	6.6
20	7.2	Instant	xxxx	−1.21	6.8
20	7.5	Opal	0	......	...

Isoelectric mixture:

5.0 millimols $AlCl_3$ in 1,000 cc.

2.663 millimols Na_2SiO_3 } 1.816 millimols Na_2HPO_4 } 5.325 millimols NaOH } in 1,500 cc.

pH 6.7.

SiO_2 in solution: 1.747 millimols.

PO_4 in solution: 0.050 millimol.

Composition of floc: $Al_2O_3(SiO_2)_{0.366}(P_2O_5)_{0.358}$.

TABLE 57B

The ferric "phospho-silicate," system no. 22

A. 5.0 millimols $FeCl_3$ in 1,000 cc.

B. 7.5 millimols Na_2SiO_3 } 5.114 millimols Na_2HPO_4 } 15.0 millimols NaOH } in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION:		μ/sec. 1 volt/cm.	pH
		After mixing	Overnight		
cc.	*cc.*				
20	6.7	Opal	xx	......	...
20	6.8	Instant	xxxx	+1.68	4.8
20	6.9	Instant	xxxx	−0.95	6.2
20	7.0	Instant	xxxx	−1.78	6.45
20	7.1	Rapid	xxxx	−2.02	6.65
20	7.2	Opal	0	......	....

Isoelectric mixture:

5.0 millimols $FeCl_3$ in 1,000 cc.

2.576 millimols Na_2SiO_3 } 1.766 millimols Na_2HPO_4 } 5.152 millimols NaOH } in 1,500 cc.

pH 6.4.

SiO_2 in solution: 2.012 millimols.

PO_4 in solution: 0.045 millimol.

Composition of floc: $Fe_2O_3(SiO_2)_{0.226}$ $(P_2O_5)_{0.[illegible]44}$.

tively from the reaction of these compounds with the humus complex, and the various mineral soil colloids show great quantitative differences among themselves. The combining power of the humus with bases is, as already stated, much greater than that of the mineral soil complex, even when the latter is highly silicated. The humus is therefore more highly electronegative and requires more of an electropositive component for electrical neutralization.

The mineral soil complexes vary greatly in "electronegativity" depending upon the degree to which they are silicated or, more generally, upon the degree

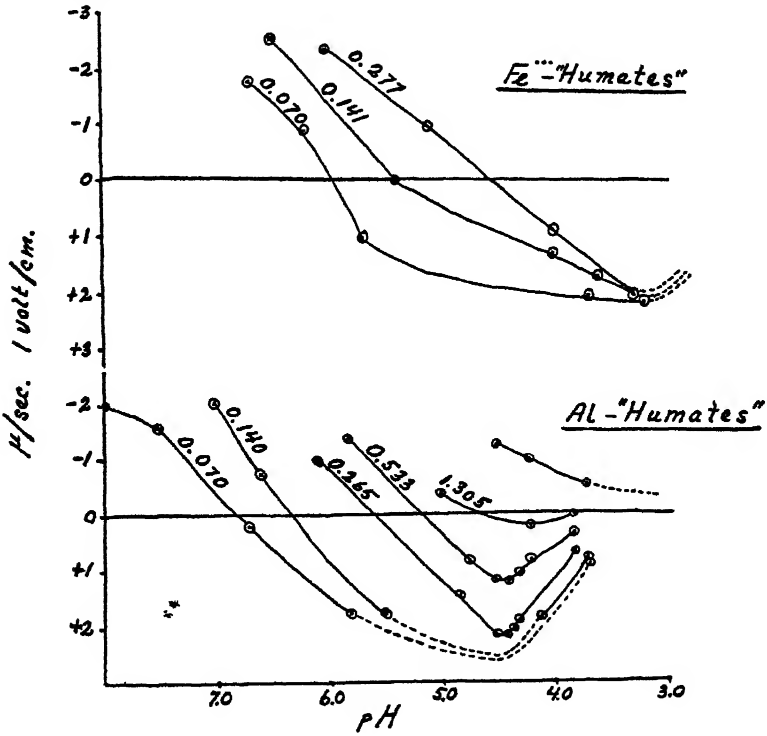


FIG. 8. THE RELATION BETWEEN THE pH, THE SIGN OF CHARGE, THE MIGRATION VELOCITY, AND THE RATIO OF GRAM HUMUS TO MILLIMOL SESQUIOXIDE IN A SERIES OF ALUMINUM AND FERRIC "HUMATES"

to which acid radicals exist in (partial) combination in the insoluble alumino or ferric complex. If highly silicated, the soil complex will precipitate isoelectrically, at a given pH, with a much greater porportion of sesquioxide than if it is only slightly silicated. The highly silicated complexes are not by themselves amphoteric but require, like the humus complex, a definite proportion of sesquioxide to form an amphoteric complex. With the minimum quantity of sesquioxide the isoelectric pH is low, but becomes progressively higher as the proportion of sesquioxide is increased, just as in the case of the humus complex. Soil

complexes of a low silication, e.g., a silica/sesquioxide ratio below 2.0, are by themselves amphoteric and any introduction of additional sesquioxide will only result in a higher isoelectric pH.

In order to show the general behavior of the sesquioxide-soil colloid systems, and for future reference in connection with the laws governing ion adsorption

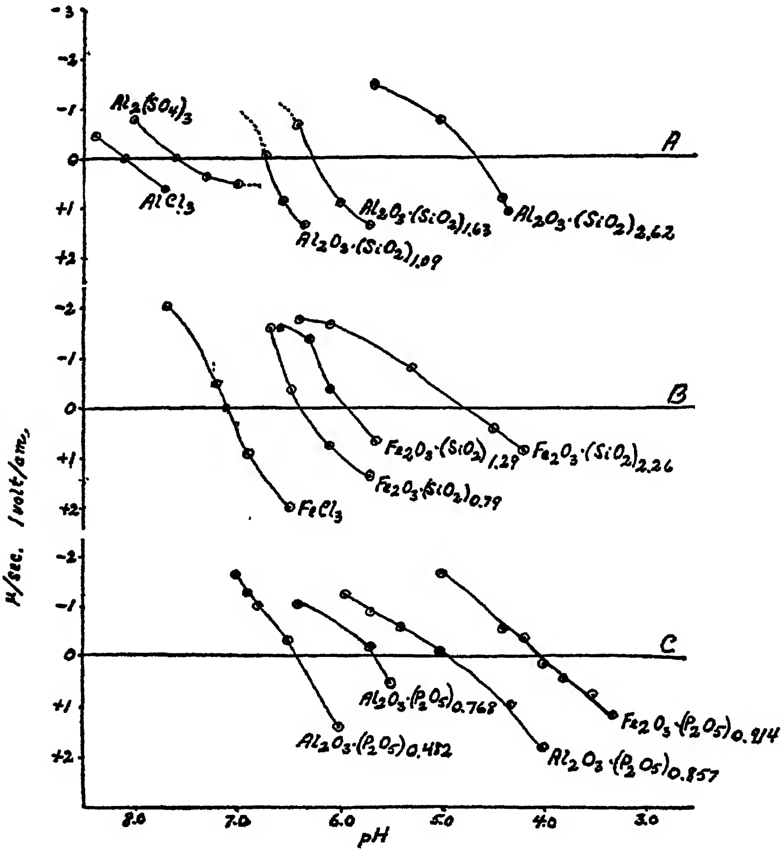


FIG. 9. THE RELATION BETWEEN THE pH, THE SIGN OF CHARGE, AND THE MIGRATION VELOCITIES OF "HYDROXIDES," "SILICATES," AND "PHOSPHATES" OF ALUMINUM AND IRON

The "silicate" and "phosphate" formulas refer to the isoelectric composition.

and exhange, table 58a and b are here appended. Bentonite was selected because of its strong electronegative character and high cation exchange capacity. One millimol sesquioxide was precipitated with 0.5 gm. bentonite, the isoelectric pH being 6.75 in the $AlCl_3$ system and 6.5 in the $FeCl_3$ system. Comparing this to the figures in table 50 were the proportion of humus is nearly the same (0.533 gm. per millimol Al_2O_3) we see that the "humated" sesquioxide

TABLE 58A

The $AlCl_3$ + bentonite, system no. 101

A. 4.0 millimols $AlCl_3$ in 1,000 cc.
B. 1.0 gm. bentonite in 1,000 cc.

SOLUTION A	SUSPENSION B, 20 cc + NaOH MILLIMOLS	FLOCCULATION:		μ/sec. 1 volt/cm.	pH
		After mixing	Overnight		
cc.					
20	0.28	0	x	−2.44	...
20	0.27	Rapid	xx	−1.96	...
20	0.25	Instant	xxxx	−1.54	7.8
20	0.24	Instant	xxxx	−1.33	7.3
20	0.23	Instant	xxxx	−0.38	6.85
20	0.22	Instant	xxxx	+0.40	6.6
20	0.21	Instant	xxxx	+1.01	6.3
20	0.20	Slow	xxxx	+2.04	5.8
20	0.18	0	x	+2.10	4.6
20	0.16	0	x	+2.03	4.4
20	0.10	Slow	xxxx	+1.79	4.2

Isoelectric mixture:
4.0 millimols $AlCl_3$ in 1,000 cc.
1.0 gm. bentonite + 11.75 millimols NaOH in 1,500 cc.
pH 6.75.
Al_2O_3 in solution: None.

Composition of floc: $\frac{1 \text{ gm. bentonite}}{2 \text{ millimols } Al_2O_3}$ = 0.5 gm. bentonite per millimol Al_2O_3.

TABLE 58B

The $FeCl_3$ + bentonite, system no. 102

A. 4.0 millimols $FeCl_3$ in 1,000 cc.
B. 1.0 gm. bentonite in 1,000 cc.

SOLUTION A	SUSPENSION B, 20 cc. + NaOH MILLIMOLS	FLOCCULATION:		μ/sec. 1 volt/cm	pH
		After mixing	Overnight		
cc.					
20	0.28	0	x	−2.34	...
20	0.27	Slow	xx	−2.10	...
20	0.26	Rapid	xxx	−1.90	8.0
20	0.25	Instant	xxxx	−1.60	7.3
20	0.24	Instant	xxxx	−1.02	6.9
20	0.23	Instant	xxxx	±0.0	6.5
20	0.22	Instant	xxxx	+1.05	5.95
20	0.21	Instant	xxxx	+1.79	5.4
20	0.20	Rapid	xxx	+2.06	4.1
20	0.18	Slow	xx	+2.16	3.7
20	0.16	Slow	x	+2.18	3.2
20	0.10	Rapid	xx	+1.96	...

Isoelectric mixture:
4.0 millimols $FeCl_3$ in 1,000 cc.
1.0 gm. bentonite + 11.5 millimols NaOH in 1,500 cc.
pH 6.5.
Fe_2O_3 in solution: None.

Composition of floc: $\frac{1 \text{ gm. bentonite}}{2 \text{ millimols } Fe_2O_3}$ = 0.5 gm. bentonite per millimol Fe_2O_3.

has a much lower isoelectric pH (5.15) than the "bentonated" sesquioxide. At the same pH the quantity of bentonite electrically neutralized by the aluminum would be very much greater than the quantity of humus so neutralized. The quantities would doubtless be in inverse proportion to the cation exchange capacity.

We have employed the terms "silicates," and "humates," keeping in mind that the colloidal complex does not represent a combination of any one pair of

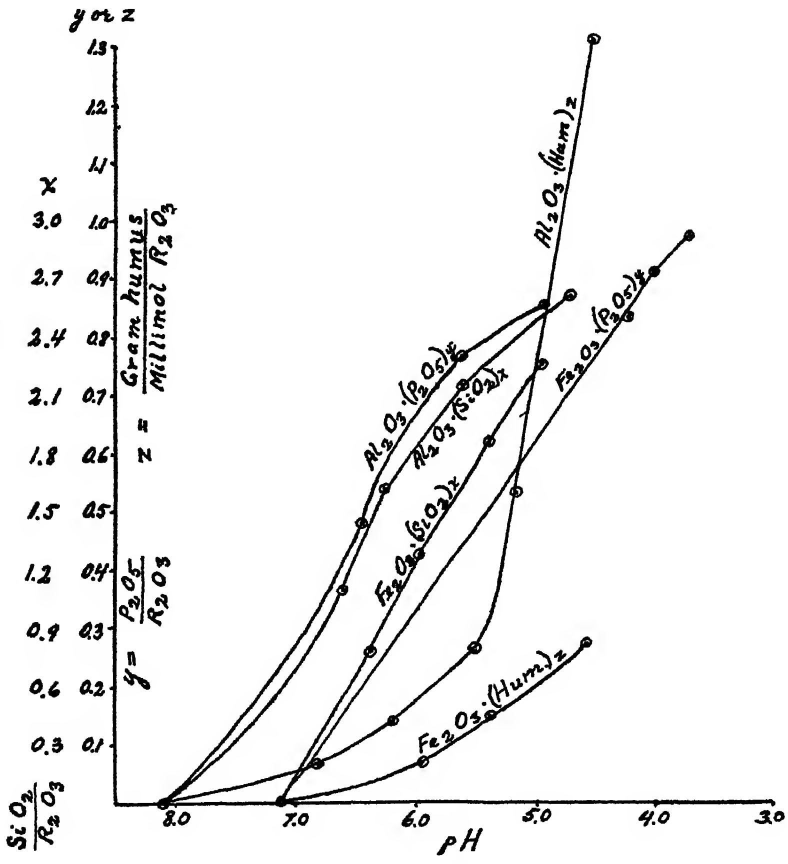


FIG. 10. THE RELATION BETWEEN THE pH AND THE ISOELECTRIC COMPOSITION OF ALUMINUM AND FERRIC "PHOSPHATES," "SILICATES," AND "HUMATES"

ions alone. The reaction between bentonite and the salts of aluminum and iron is apparently of the same nature as the reaction of these salts with the silicate and humate of sodium. The bentonite plays the part of the acid radical, but it would obviously be meaningless to call the resulting complexes "bentonates." There can be no doubt that the Al and Fe valences partly interact with the free silicate valences in the bentonite. The new complex repre-

sents then a less silicated complex than that of the original bentonite. This will be brought out in the studies on ion adsorption and exchange.

SUMMARY

This, the second paper, on isoelectric precipitates, deals with the "humates," "phospho-humates," "silico-humates," and "phospho-cilicates" of aluminum and iron.

The "humates" differ from the "silicates" and "phosphates" in that the colloidal humic complex is quantitatively precipitated by the $AlCl_3$ and $FeCl_3$, in showing an electropositive maximum on the acid side of the isoelectric point and, where the proportion of humus is high, in having a second isoelectric point.

The ferric "humates" are isoelectric at a much lower pH than the aluminum "humates" of corresponding composition.

The humate ion, or ion complex, displaces the PO_4 ion and, to a still greater extent, the SiO_3 ion. The PO_4 ion strongly displaces the SiO_3 ion. There is no apparent displacement of the PO_4 ion by the SiO_3 ion, for in the presence of the latter the proportion of the P_2O_5 in the complex increases somewhat. This anomaly may be due to the formation of an addition complex and will be discussed later in connection with ion adsorption and exchange.

The mineral soil colloid complexes react with aluminum and iron, forming isoelectric precipitates in exactly the same manner as the humus complex.

REFERENCES

(1) BAUMANN, A., AND GULLY, E. 1910 Untersuchungen über die Humussäuren. *Mitteil. Bayer. Moorkulturanst.* 4: 31–156.

(2) CLARKE, F. W. 1911 The Data of Geochemistry. Washington, D. C.

(3) GHOSH, J. C., AND BHATTACHARYYA, P. B. 1930 Removal of ions from solutions of calcium dihydrogen phosphate by treatment with hydrous gels of alumina, silica and their mixtures. *Soil Sci.* 29: 311–322.

(4) MATTSON, SANTE 1928 Cataphoresis and the electrical neutralization of colloidal material. *Jour. Phys. Chem.* 32: 1532–1552.

(5) MATTSON, SANTE 1928 The electrokinetic and chemical behavior of the alumino-silicates. *Soil Sci.* 25: 289–311.

(6) ODÉN, SVEN 1919 Die Huminsäuren. *Kolloidchem. Beihefte* 11: 75–260.

(7) TAMM, OLOF 1920 Soil studies in the needle forest region of northern Sweden. *Meddel. Statens Skogsförsöksanst* [*Sweden*] 17: 49–300.

(8) TRUOG, E. 1929 The origin, nature, and isolation of the inorganic base exchange compound of soil. *Jour. Amer. Soc. Agron.* 21: 1030.

BOOK REVIEWS

Grundzüge der Bodenkunde. By FRIEDRICH SCHUCHT. Paul Parey, Berlin, 1930. Pp. VII + 405, figs. 135.

The author, who is director of the Institute of Geology, Mineralogy, and Soils of the University of Berlin, notes that the data of soil science are of interest not only to students of agriculture and forestry, but also to those of geology, botany, and economic geography. The author wrote his book with this in mind.

The major divisions of the book include soils and soil science, soil formation, soil physics, the chemistry of soils, the biology of soils, influence of climate on soil formation, the soil as a culture medium for plants, soil classification and mapping, the soils of Germany, and methods of soil examination.

The author's style is clear and the discussion is usually compact. Despite the briefness of the treatment made necessary by the character of the book, the discussion of the various topics is usually effective. For example, the author's description of the formation of lateritic soils, of peat soils and their formation, of base exchange and soil acidity, is quite satisfactory. There is also a lucid discussion of climate in its relation to soil formation. The portion dealing with the soils of Germany will be of particular interests to teachers located in other countries. The bibliography is arranged under various topics and is quite adequate for a book of this character.

Die Bedingungen der Wirtschaftlichkeit der Handelsdüngemittel. By L. VON KREYBIG. Paul Parey, Berlin, 1930. Pp. 128, figs. 5, colorplate 1.

The book is dedicated to Prof. F. Löhnis, of the University of Leipsic, and contains a foreword by him.

The topics dealt with in the book include chemical factors which influence the action of fertilizers; the influence of microörganisms on the action of manures and fertilizers; the plant physiological factors affecting the action of fertilizers; conditions relating to the application and economic returns from nitrogenous fertilizers, phosphates, potash fertilizers, and mixed fertilizers; and practical considerations in the use of fertilizers.

The author develops some interesting points of view concerning the plant-food resources of the soil, the supplementing of these with plant-food derived from animal manures and chemical fertilizers, the significance of microörganisms in the circulation of plant-food, and the economic considerations that enter into the utilization of the plant-food resources derived from within or without the soil.

Manual of Bacterial Plant Pathogens. By CHARLOTTE ELLIOTT. The Williams and Wilkins Company, Baltimore, Md., 1930. Pp. IX + 349.

The author, who is associate pathologist in the Bureau of Plant Industry, U. S. Department of Agriculture, has arranged her treatise in four major divisions; namely, Alphabetical List of Plant Pathogens, Alphabetical List of Non-Pathogenic Organisms Associated with Plant Disease, Chronological Chart of Plant Pathogens, and Index of Host Plants and Bacteria.

The individual species of plant pathogens are described in the first section, in each case the name of the organism, synonymous name, symptoms, host plant, geographical distribution, and literature are recorded. This portion of the book occupies 270 pages out of a total of 349. A similar treatment is accorded to non-pathogenic organisms associated with plant diseases (p. 273–317). The chronological charts of plant pathogens (p. 320–331) offer convenient reference material. The book will undoubtedly be appreciated not only by plant pathologist, but also by botanists generally, microbiologists, and horticulturists.

Mycorrhiza. By M. C. RAYNER. Wheldon & Wesley, Ltd., London, 1927. Pp. VI + 246, figs. 64.

The increasing interest in soil biology has served to attract a greater degree of attention to mycorrhiza. Much has been published on the subject, but, unfortunately, the material is scattered in various journals and reports and not always readily accessible to the student. For this reason, the author has rendered a helpful service in having brought together much of the scattered material. She has organized the data and has presented them in a very readable way. As has been noted by the author:

> The mycorrhiza habit first attracted attention as a characteristic of woodland soils. Modern experimental research has once more focused attention on the significance of this fact and emphasized the importance of mycorrhiza as a biologic soil factor affecting the vegetation of woodlands in common with that of other types of humus soil. The practical application of the newer knowledge to natural conditions has already attracted the attention of foresters, thus opening up a new and fascinating field of research, promising alike to the botanist, the forester and the student of soil science.

The book is divided into three parts, dealing, respectively, with the early period (1840-1880), the second period (1880 to about 1900), and the modern period (1900–1925). There are, in all, 11 chapters. These deal with the various hypotheses and controversies that have centered about the subject of mycorrhiza. The reader will find among the multitude of topics discussed: the Monotropa controversy, De Bary's theory of symbiosis; the morphological studies on mycorrhiza by Frank; Hartig's theory of parasitism; the contributions of various workers on the subject of saprophytes and hemisaprophytes; the cytology of intracellular digestion; and autotrophic and mycotrophic plants. There is a discussion of mycorrhiza of Arctic and Alpine plants; of orchidaceae; ericaceae; endotrophic and ectotrophic mycorrhiza; "mycorrhiza"

in bryophyta; tuberisation; and the physiological significance of mycorrhiza and the nutrition of mycorrhiza plants. A very satisfactory bibliography has been supplied by the author.

Handbuch der Bodenlehre, volume II. Edited by E. BLANCK. Julius Springer, Berlin, 1929. Pp. VI + 314, figs. 50.

The first volume of the Handbuch (reviewed on page 260, volume XVIII of SOIL SCIENCE) deals with the scientific principles relating to the origin of soils. The second volume deals with the weathering of rocks and the climatological processes of weathering. The contributors to the first volume, aside from the editor, include Fesefeldt, Giesecke, Hager, Heide, Meigen, Passarge, Philipp, Rehorst, and Rüger. The contributors to the second volume include, aside from the editor, Knoch, Rehorst, Schellenberg, Schubert, and Wasmund.

The principal divisions of the book deal with factors of climate and climate zones; the climate of the soil surface and of the lower portion of the atmosphere in Central Europe; variation in climate in recent geological periods; pollen studies as a means of measuring climatological factors of recent geological periods and of the age of humus formations; general facts about weathering; physical factors of weathering; chemical factors of weathering; the decomposition of organic substances; biological weathering due to living organisms; and biological weathering as influenced by organic substances undergoing decomposition.

The present volume contains important contributions to our knowledge of climate as a major factor in the genesis and evolution of soils. The extensive bibliography is both suggestive and helpful, and should enable the student of soils, as well as the ecologist and plant physiologist, to understand more clearly the influences that have played a part in the past, as they now do, in maintaining dynamic conditions in the soil.

Klima und Boden in ihrer Wirkung auf des Pflanzenleben, second edition. By HENRIK LUNDEGÅRDH. Gustav Fischer, Jena, 1930. Pp. x + 480, figs. 129, maps 2.

There is an evident tendency among workers in the fields of plant and soil science to organize the pertinent facts in such fashion as to permit of more sound and effective generalizations. The present volume is a good illustration of this tendency. This treatise on climate and soil in their relation to plant life is the result of the author's painstaking study in various fields. As he himself expresses it:

> The field of experimental ecology, new and yet rapidly expanding, is criss-crossed with so many paths of research and so many trends of thought that its description, if it is not to exceed certain limits, must confine itself to essentials only. I have tried to extract much from the most recent literature on the subject. Nevertheless, I have considered it desirable to lay emphasis on those parts that are related to my own experimental inquiries.

A brief historical discussion is followed by chapters on the light factor, the temperature factor, and the water factor. Other chapters deal with the soil, its formation and general ecological properties, the physical nature and the aeration of soils, the chemical soil factors, soil microörganisms, the carbonic acid factor, and the more important principles which relate to experimental ecology.

The author is to be commended for the logical arrangement and discussion of the various topics, the helpful footnotes, the rather satisfactory bibliography, and the carefully prepared index. The publishers deserve credit for the attractive appearance of the book.

Bacteriology, second edition. By STANLEY THOMAS. McGraw-Hill Book Company, Inc., New York, 1930. Pp. XV + 301, figs. 38.

The author, who is professor of bacteriology at Lehigh University, has written a textbook which attempts to outline in a balanced way the more important fields of general bacteriology. In the introduction to the first edition, which was published in 1925, he noted that

> While medical bacteriology has been of invaluable service to mankind, and while there is no doubt that the impetus given by Pasteur, Koch and Lister has put this branch of the science ahead of the other branches in pure achievement, the fact must not be lost sight of that medical bacteriology is simply one branch of a science which is, like its parent sciences chemistry and biology, of educational and practical value to mankind in many and varied fields of endeavor.

In the second edition, which was revised and rewritten, he says:

> Modifications, additions and omissions have been required in every chapter, and in most cases an entirely new method of presentation has been employed.

The book contains 17 chapters, which deal with such topics as the history of bacteriology; classifications; morphology, physiology, and cultivation of bacteria. There is also a discussion of bacterial enzymes and of microörganisms other than bacteria. Special chapters are devoted to bacteria in the soil, water, sewage, air, and foods. Three chapters are devoted to a discussion, respectively, of pathogenic bacteria, immunity, and hygiene and sanitation. The last chapter deals with bacteria in industry. The frontispiece is a good reproduction of one of Pasteur's protraits.

Ergebnisse der Agrikulturchemie, volume I. Edited by F. HONCAMP. Verlag Chemie, Berlin, 1929. Pp. VII + 281, figs. 28.

This volume was prepared at the request of the section on agricultural chemistry of the Association of German Chemists, and represents contributions from men well known among the chemists of Germany and of other countries. The introductory chapter, entitled "Chemistry and Agriculture," was written by Prof. A. Binz, of Berlin. The titles of the other chapters and the names of their authors follow: "Distribution and Circulation of Iodine from the Point of

View of its Significance in Soils," by Griessbach; "The Significance of Iodine in Plant Nutrition," by Ströbele; "The Iodine Problem in Animal Nutrition," by Scharrer; "The Combined Action of the Elements Potassium and Sodium in Plant Growth," by Maiwald; "The Cultivation and Manuring of Soils," by Alten; "Modern Chemical and Biological Points of View in the Treatment of Stable Manure," by Ruschmann; "Definite Relations in the Assimilation of Protein," by Wöhlbier; "Experiences, Practical Observations and Results Relating to Phosphoric Acid Studies in Soils," by Doerell; "The Lime Resources of our Soils," by Behrens; "On the Determination of the Saturation Point of Soil in Accordance with Newer Methods," by Kappen; "Electrodialysis and the Problem of Mineral Soil Acidity," by Trénel; "The Measurement of Soil Reaction, the Influences Modifying Soil Reaction and the Biological Appraisal of the Soil," by Görbing and Adolphi; "Results Obtained in Determining the Lime Requirements and in Modifying the Saturation Point of Soils," by Pfeil.

The book contains no index, but it is to be presumed that a general index will be included in the last volume of the series.

Soil. By ARCHER BUTLER HULBERT. Yale University Press, New Haven, 1930. Pp. 227, figs. 7.

The title of the book is somewhat misleading in that the term "soil" is used, in part at least, as being equivalent to the term "land."

In writing the book, the author had a definite objective, as is indicated in the preface. He says:

> The main theme of the present volume is the quite ignored one of the influences of the soil on American settlement and expansion. The plan of the volume, however, has been arranged in the hope of doing more than presenting merely a new and important phase of American history; it is offered as a kind of non-classroom textbook for those who may some day undertake to produce the constructive type of local history so greatly needed.

The author states further that he has tried to show "how geological, climatic, hydrographic and edaphic factors have been and may be used to clarify history, particularly the history of American occupation and expansion."

The 21 chapters deal with the basis of provinces; climatic influences on man and vegetation; the waterway keys to our soil provinces; some aspects of river control; highland pathways of conquest and migration; the story of our soils; soil and migration; the meadows of New England; the call of the Connecticut; the Nipmuck and chestnut countries; the tidewater pioneers; the Virginia Piedmont; human seed on stony ground; Penn's forest empire; Scot and Celt on the American frontier; the grand advance; beyond the Shenandoah; the conquest of the Alleghenies; the blue grass region of Kentucky and beyond; types of soil influence in the west. There is also an appendix entitled "A New Basis for the Study of Local History."

Those interested in the field of soil science, as well as economists, sociologists, and historians will find much that is suggestive and helpful in this book.

Lehrbuch der allgemeinen Bodenkunde. By ALEXANDER STEBUTT. Gebrüder Borntraeger, Berlin, 1930. Pp. XII + 518, figs. 55.

Formerly connected with soil research activities at Saratov and Moscow and now professor at the University of Belgrad in Jugoslavia, the author is intimately acquainted with the accomplishments of the Russian school of soil science. He has had also wide experience as a teacher of soils, and possesses, for this reason, a broad outlook on the whole field of soil science. In planning the book he was guided by the central thought that is clearly expressed in the preface. He says:

> Any field of science may attain unity only when its parts are tied together by a central, leading thought. This leading idea is brought to us by Soil Science in its new conception of the soil as a natural body *suis generis* whose condition is a dynamic one. Thanks to this dynamic condition, the soil is passing through the process of development and evolution as opposed to the fundamental idea in the old conception of the soil as a dead, mechanical accumulation of rock fragments and devoid of energy. This book represents the systematic development and expression of the central thought and reflects essentially, the conception of the soil as a dynamic system.

The first part of the book, entitled "The Soil Forming Substratum," contains chapters on the composition of the earth's crust, formation of klastic sediments, the porous soil mass as a disperse system, colloidal systems, soil porosity, and the relation of soils to water, air, organic substances, and heat.

The second part deals with the dynamics of soils and contains chapters on the significance of water in soil formation processes, the decomposition of minerals, the synthesis of soil compounds, soil acidity, mobility of soil constituents, impoverishment of soil layers, and enrichment of soil layers.

In the third part, the general topic of the "genesis of soils," is considered in connection with the more important changes that occur in soil formation, zones of the earth's surface and their relation to one another, plant societies and soils, variations in soil forming processes in different zones, and soil classes from the points of view of immaturity, zeolite formation, degradation.

The fourth part deals with soil fertility as the applied phase of soil science.

JACOB G. LIPMAN.

THE TEMPERATURE CORRECTION IN THE HYDROMETER METHOD OF MECHANICAL ANALYSIS OF SOILS

CHARLES RICHTER

Hawaii Agricultural Experiment Station

Received for publication April 24, 1930

The employment of the hydrometer for the measurement of soil-suspension concentrations in the course of mechanical analysis has become quite widespread since its proposal by Bouyoucos in 1927. The method has been found valuable by many investigators for routine analysis in soil surveys and for soil classifications.

The method, which is said to be based on heat of wetting determinations, has been checked by several investigators against other methods such as the pipette method and the water-vapor adsorption method. It appears that the hydrometer method agrees fairly well with the pipette method, but not so well with the water-vapor adsorption method in the case of soils of high organic matter content. This latter fact of course does not discount the value of the hydrometer method; it simply means that with certain soils it seems inadvisable to attempt to translate physical properties into particle size or vice versa.

The hydrometer method is too well known to be described here again in detail, and for this reason only a very short resumé is given.

An aliquot of soil (25, 50, 75, or 100 gm.) is dispersed, in a specially constructed metal cup fitted with buffers, by an electric mixer for a required length of time with the addition of 5 cc. *N* KOH solution. After the dispersion has been completed the contents are transferred to a glass cylinder, made up to a definite volume with distilled water, shaken, the hydrometer placed into the soil suspension, and the mixture allowed to settle. After 15 minutes the hydrometer readings which give the amount of soil in grams left in suspension, are taken. This is considered as the colloidal fraction. The time for complete dispersion as recommended by Bouyoucos is 9 minutes, which is probably sufficient for many mainland soils but very insufficient for heavy lateritic or organic soils. For most Hawaiian soils at least 30 minutes dispersion is required, in certain cases 60 minutes. In this investigation 30 minutes dispersion was used in every case. The water used was 1,000 cc. plus as many cubic centimeters more as there were grams of soil. The length of time required for settling assumes a uniform specific gravity of about 2.65 for soils; for a more exact and detailed mechanical analysis the settling time must be calculated from the specific gravity of soils, the temperature of the mixture, and the height of the column of suspension. Since the hydrometer is calibrated to give correct readings at 67°F., under or above this temperature a necessary correction is made, i.e., 0.35 is added to the reading for each degree above 67°F., and subtracted for each degree below 67°F. The corrected reading divided by the weight of soil used gives the percentage of fraction in question.

Although the practical usefulness of the hydrometer method is recognized, it is desirable to know the degree of accuracy of which it is capable at low range as well as at high range. Is the same correction to be applied to the readings at all concentrations and at all temperatures? It is evident from fundamental considerations that the temperature correction of 0.35 for each degree cannot hold true for the whole range of the hydrometer and that the error introduced becomes greater as the concentration becomes lower. Moreover, it may be anticipated that at any one concentration the temperature correction at different temperatures will not be a straight line function but a curve.

TABLE 1

*Relation of concentration of soil suspension to the error involved in the application of the temperature correction of ± 0.35 factor to the hydrometer reading in case of a typical Hawaiian clay**

AMOUNT SOIL USED	HYDROMETER READING AT 67°F.	HYDROMETER READING AT 87°F.	COLLOIDS ISOLATED	COLLOIDS PER GM. SOIL CALCULATED FROM READING AT 67°F.	COLLOIDS CALCULATED FROM READING AT 87°F. USING 0.35 FACTOR	COLLOIDS PER GM. SOIL CALCULATED FROM WEIGHT OF COLLOIDS	ERROR INVOLVED DUE TO TEMPERATURE CORRECTION	COMPUTED CORRECTIONS TO GIVE APPROXIMATELY CORRECT RESULT
(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
gm.			*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	
100	63.0	56.0	65.7	63.0	63.0	65.7	0.0	0.35
80	50.5	45.0	51.3	63.1	65.6	64.1	4.1	0.27
60	37.5	33.0	38.5	62.5	66.7	64.1	5.9	0.24
40	24.5	21.0	24.9	61.2	68.8	62.2	9.2	0.21
20	12.0	9.0	11.4	60.0	80.0	57.0	27.0	0.18
10	6.0	3.5	6.1	60.0	105.0	61.0	66.7	0.14
5	3.0	0.5	3.0	60.0	150.0	60.0	138.1	0.13
Average......						62.9†	.....	.....

* Figures expressed in terms of oven-dry soil.
† Omitting fifth number, for 20 gm. of soil.

In the following experiments, the effect of concentration and temperature of the soil suspension on the accuracy of the hydrometer method has been investigated, both mainland and Hawaiian soils, high in clay fraction, being used.

The soils used in this investigation were as follows:

1. Light grayish-brown river-bottom clay from Arlington farm, Virginia. Specific gravity, 2.62; organic matter, 5.72 per cent; pH, 5.30.
2. Light-brown Pacific Coastal soil from Bellingham, Wash. Specific gravity, 2.54; organic matter, 3.21 per cent; pH, 5.70.
3. Light-brown clay from Lanikai, Oahu, Hawaii. Specific gravity, 2.83; organic matter, 3.94 per cent; pH, 8.23.
4. Yellowish-brown clay from Hakalau, Hawaii. Specific gravity, 2.84; organic matter, 9.07 per cent; pH, 6.50.
5. Brownish-red clay from Maui, Hawaii. Specific gravity, 2.96; organic matter, 3.27 per cent; pH, 7.60.

Table 1 shows the relation of the soil-suspension concentration to the error involved in the application of the temperature correction of plus-minus 0.35 factor to the hydrometer reading in the case of a typical Hawaiian clay (soil 5). Various amounts of soil (column A) in simple multiples (5, 10, 20, 40, 60, 80, 100 gm.) were dispersed as described in the foregoing and brought to the required volume. The suspension was cooled down to 67°F., shaken and placed into a water-bath adjusted to 67°F., and allowed to settle for 15 minutes. At 15 minutes the hydrometer reading was taken (column B). The cylinder

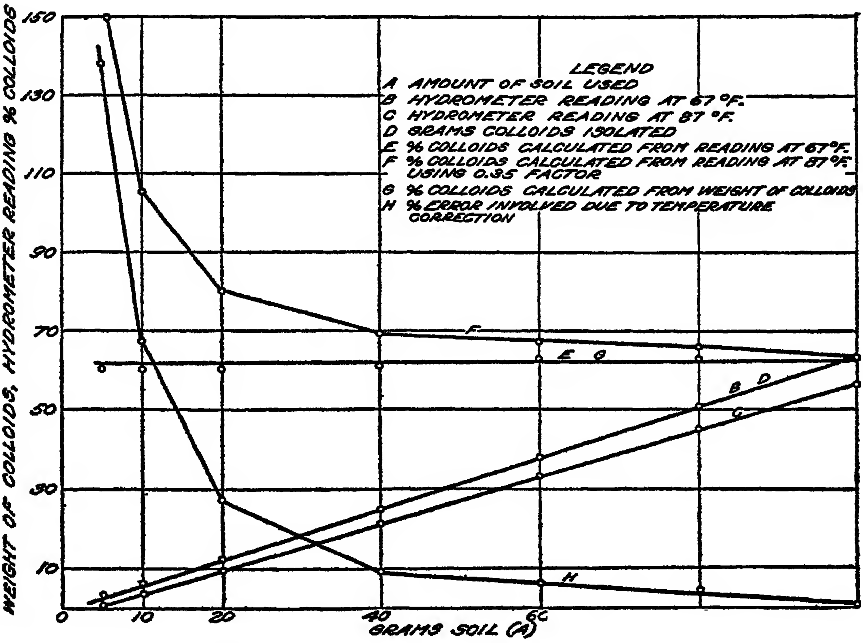


Fig. 1. Relation of Concentration of Soil Suspension to the Error Involved in the Application of the Temperature Correction of the ± 0.35 Factor to the Hydrometer Reading

with its contents was placed into the thermostat for overnight at 87°F. The hydrometer reading was again taken after 15 minutes settling at 78°F. (column C), and the soil suspension immediately siphoned off to the point where the solid matter was settling. The suspended solid matter was isolated by evaporating to dryness on the water-bath. The obtained colloids were then dried at 125°C. for 18 hours and weighed (column D). Column H was obtained by subtracting 63 (the assumed correct value) from the value given in column F and dividing by 0.63. The last column was obtained by the following formula: $\frac{0.63A\text{-}C}{20}$. These data are arranged graphically in figure 1. As may be seen from the foregoing the agreement between the weight of isolated

colloids and the hydrometer reading at 67°F. is good, but that the error involved in calculating the percentage of colloids from the hydrometer reading at 87°F. becomes considerable at lower concentrations when the same temperature factor of plus-minus 0.35 for each degree F. is used throughout. In this set of figures it was assumed that the correct percentage of colloids is 63, i.e., the reading at 67°F. of the soil suspension when 100 gm. of soil was used. This figure agrees with the over-all average of the percentages of colloids calculated from the weight of colloids isolated for all concentrations (average of column G).

If the computed corrections at different concentrations (i.e., the values appearing in column I) are plotted against the corresponding hydrometer readings at 87°F. and connected with a smooth curve (fig. 2), it becomes apparent that the correction to be applied for each degree F. decreases steadily from the upper to the lower range of the hydrometer.

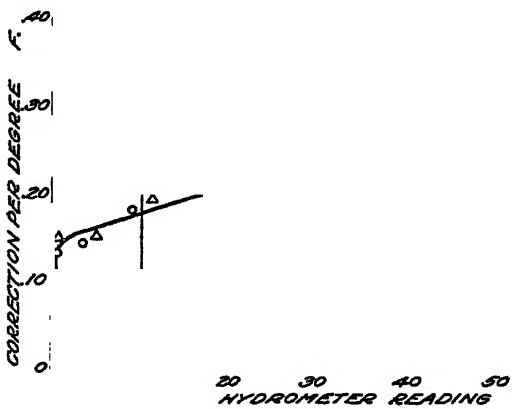


FIG. 2. CORRELATION BETWEEN HYDROMETER READING AND COMPUTED CORRECTION TO GIVE CONCORDANT RESULTS

It is to be noted that a very good parallelism obtains between the hydrometer reading at 67°F. and 87°F. when they are plotted against the concentrations.

In order to see the correlation between hydrometer reading and increasing temperatures at different concentrations, soil suspensions were made at three different concentrations (10, 50, and 100 gm. to a liter of water) with a number of soils, and their apparent density was successively determined by means of the hydrometer at increasing temperatures after 15 minutes settling. The soil suspension was cooled down to about 50°F., shaken, and allowed to settle for the required time, when the hydrometer and temperature readings were taken. This procedure was repeated several times at different temperatures as the soil suspension was warming up. After the last reading, which was taken at about room temperature (84–88°F.), the cylinder with its contents was placed

TABLE 2

Correlation between hydrometer reading and temperature at different concentrations, and the computed corrections

SOIL NUMBER	CONCENTRATION OF SOIL SUSPENSION								
	10 gm. per liter water			50 gm. per liter water			100 gm. per liter water		
	Hydrometer reading	Temperature	Computed correction per degree F.	Hydrometer reading	Temperature	Computed correction per degree F.	Hydrometer reading	Temperature	Computed correction per degree F.
		°F.			°F.			°F.	
1	5.5	49.5	−0.06	17.5	48.0	−0.11	31.5	46.0	−0.13
	5.2	56.0	−0.06	17.0	56.0	−0.14	31.0	52.0	−0.15
	5.0	60.0	−0.07	16.5	60.0	−0.14	30.5	58.0	−0.19
	4.5	67.0	*	16.0	63.5	−0.14	29.8	62.0	−0.20
	3.8	74.5	+0.09	15.5	66.5	*	29.2	64.5	−0.16
	3.0	81.5	+0.10	15.2	68.5	+0.13	28.5	67.5	*
	...	....	......	14.5	74.0	+0.14	28.0	71.0	+0.20
	...	....	......	13.5	79.5	+0.16	26.5	77.5	+0.22
	...	....	......	11.5	87.0	+0.20	25.5	82.5	+0.21
	...	....	......	....	....	......	24.0	87.0	+0.24
2	6.0	50.0	−0.07	19.0	50.0	−0.16	33.5	52.0	−0.20
	5.8	56.0	−0.09	18.5	56.0	−0.20	32.5	58.0	−0.22
	5.5	62.5	−0.14	17.5	61.0	−0.20	31.2	63.5	−0.20
	4.7	68.0	*	16.5	66.0	−0.20	30.5	67.0	*
	3.5	75.0	+0.16	15 5	73.0	+0.16	30.0	69.0	+0.25
	2.5	81.5	+0.16	14.0	79.0	+0.19	29.0	73.0	+0.25
	1.5	87.5	+0.16	12.5	86.0	+0.20	27.5	79.0	+0.25
	...	....	......	....	....	......	26.0	84.0	+0.26
3	8.5	55.0	−0.10	33.0	52.0	−0.10	62.0	52.0	−0.19
	8.0	60.5	−0.11	32.5	58.0	−0.11	61.0	58.5	−0.21
	7.5	66.0	*	32.0	62.5	−0.11	60.0	63.0	−0.20
	6.5	73.0	+0.13	31.5	67.0	*	58.7	69.0	+0.25
	5.5	80.0	+0.14	30.5	74.0	+0.14	58.0	72.5	+0.22
	4.3	86.0	+0.16	29.0	80.0	+0.19	56.5	78.0	+0.25
	...	....	......	27.0	86.0	+0.24	55.3	82.0	+0.26
	...	....	......	....	....	......	54.7	84.5	+0.26
4	8.0	51.0	−0.08	32.0	51.0	−0.11	62.5	53.0	−0.13
	7.5	59.0	−0.10	31.2	59.0	−0.11	61.7	60.0	−0.14
	7.0	65.0	−0.15	30.5	66.0	−0.20	61.0	66.0	*
	5.5	75.0	+0.15	28.5	76.0	+0.20	58.5	76.0	+0.24
	3.0	89.0	+0.17	26.0	85.0	+0.24	56.5	83.0	+0.26
5	8.0	55.5	−0.10	35.5	51.0	−0.16	64.5	50.0	−0.17
	7.5	62.5	−0.13	34.3	59.0	−0.16	62.7	62.0	−0.22
	7.0	66.5	*	33.5	65.0	−0.25	61.5	66.0	*
	6.0	74.0	+0.13	32.5	69.0	+0.25	60.0	73.0	+0.27
	4.5	80.5	+0.18	31.5	73.0	+0.25	58.5	80.0	+0.24
	3.0	86.0	+0.21	30.0	80.0	+0.23	57.0	86.0	+0.24
	...	....	......	27.0	91.0	+0.25	....	....	......

The weight of isolated colloids for the above soils were as follows for the 10, 50, 100 gm. per liter concentrations respectively: Soil 1, 3.3, 13.55, 31.2 gm.; soil 2, 3.2, 14.5, 33.1 gm.; soil 3, 6.1, 31.3, 66.3 gm.; soil 4, 5.7, 27.9, 57.7 gm.; soil 5, * * * (not determined).

into a thermostat and was allowed to come to a constant temperature (86°F.), when it was shaken up and allowed to settle for 15 minutes. The suspension was then siphoned off to the point where the solid matter was settling, and the colloids were separated as described in the foregoing and weighed. By plotting the hydrometer readings against the corresponding temperatures and drawing the curve, one obtains the correct value at 67°F. The difference between this

TABLE 3

Calculated average hydrometer readings for all soils and the corresponding computed corrections

CONCENTRATION PER LITER WATER	TEMPERATURE	HYDROMETER	CORRECTION PER DEGREE F.
gm.	*°F.*	*°F.*	
10	50	7.35	−0.08
	55	7.05	−0.08
	60	6.75	−0.10
	65	6.30	−0.12
	67	6.05	......
	70	5.50	+0.13
	75	4.95	+0.14
	80	4.10	+0.15
	85	3.10	+0.16
50	50	27.45	−0.12
	55	26.95	−0.13
	60	26.35	−0.14
	65	25.65	−0.15
	67	25.35	......
	70	24.75	+0.20
	75	23.85	+0.19
	80	22.75	+0.20
	85	21.35	+0.22
100	50	50.95	−0.17
	55	50.30	−0.19
	60	49.45	−0.21
	65	48.45	−0.22
	67	48.00	......
	70	47.40	+0.20
	75	46.25	+0.22
	80	45.00	+0.23
	85	43.65	+0.24

value and the various hydrometer readings, divided by the difference between 67°F. and the observed temperature, gives the correction that has to be applied for each degree F. to obtain the correct value (table 2).

For hydrometer readings which were very near 67°F. i.e., within one degree above or below, the correction has not been computed, because in these cases the experimental error involved in both the hydrometer and temperature reading may cause too great a deviation in computing the correction.

The values given in table 2 were averaged for all soils and are given in table 3. These composite averages are also shown graphically in figure 3, where the relation of the density of water at different temperatures to the hydrometer reading at different temperatures is also given.

Several things become apparent from these data. As is seen, the form of curve obtained when the hydrometer readings are plotted against the cor-

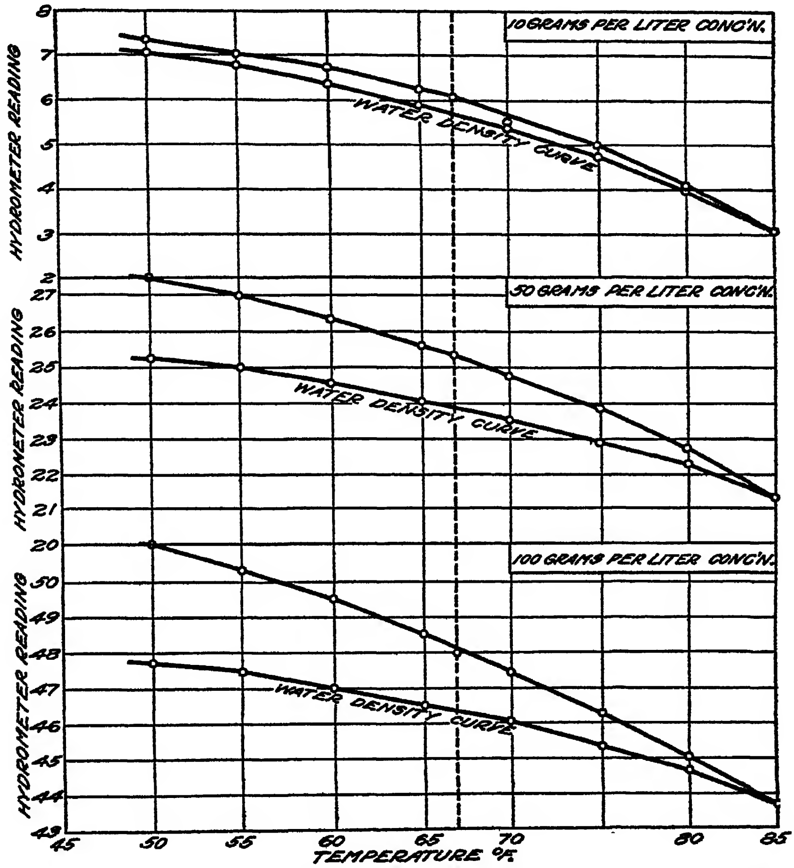


FIG. 3. CORRELATION BETWEEN TEMPERATURE AND HYDROMETER READING AT DIFFERENT CONCENTRATIONS AND THEIR RELATION TO THE DENSITY OF WATER AT DIFFERENT TEMPERATURES

Composite average of five soils

responding temperatures resembles closely that of the density of water when plotted against the temperatures. The slope of the hydrometer curve approaches that of the water-density curve at the low range of the hydrometer.

The corrections obtained for each degree F., as shown in the last column of table 3, indicate that: (*a*) at any one temperature the correction to be applied

varies with the hydrometer reading, i.e., it becomes smaller as the hydrometer reading decreases, as was shown in table 1; (*b*) at any one concentration the correction to be applied varies with the temperature, i.e., the correction increases as the temperature increases; (*c*) the correction for each degree F. below 67°F. is smaller than above 67°.

As is seen, the correction obtained for each degree F. at 85°F., as shown in the last column of table 3 (i.e., +0.16, +0.22, and +0.24 respectively) agrees well with the corrections obtained at that range and approximately at that temperature in the case of soil 5, as given in table 1.

From the foregoing it becomes apparent that the application of the temperature correction factor of 0.35 for each degree F. does not hold true at all hydrometer readings and at all temperatures, but that a sliding scale correction table is desirable with the method to increase its accuracy. The sliding scale table suggested tentatively in table 1 and figure 2 may not hold true exactly for all soil types and is probably subject to modifications, but nevertheless it indicates the nature of the rule which has to be applied in the construction of these tables of corrections.

SUMMARY

The rôle of concentration of soil suspension and temperature in the correction factor of the hydrometer method of mechanical soil analysis has been investigated.

Soil suspensions of the same soil at different concentrations but at the same temperature will not give identical results when the temperature correction factor of 0.35 is applied at all concentrations.

The same soil suspension which gave a certain reading at 67°F. will not give the same result at a higher or lower temperature if the temperature correction factor of 0.35 is applied unconditionally.

In order to obtain concordant results, the correction to be applied for each degree F. becomes considerably smaller at the lower range of the hydrometer than at the higher range.

The corrections to be applied below 67°F. are different from those to be applied above 67°F.

The nature of the curve obtained when hydrometer readings are plotted against the corresponding temperatures is graphically shown.

The sliding scale nature of a correction table to give concordant results at all concentrations is pointed out.

A sliding scale correction table is tentatively suggested, subject to modification.

INTERACTION BETWEEN AMMONIA AND SOILS, AS A NEW METHOD OF CHARACTERIZING SOIL COLLOIDS

AMAR NATH PURI

University Chemical Laboratory, Lahore, India

Received for publication May 1, 1930

In earlier publications (4, 5) the author has shown that the so-called adsorption of ammonia by soils is a chemical reaction representing the neutralization of acidoid by a base. Methods of estimating soil colloids based on ammonia absorption therefore cannot be employed for the purpose intended. It seemed, however, that this reaction could be used for finding the base neutralizing power of acidoids in different soils (6). The object of this investigation was to explore the possibilities of this reaction as a means of differentiating between soil colloids from various sources.

Interaction between bases and soil acidoids, is a slow process; and several days may be required for equilibrium to be reached. This is shown in a striking manner in table 1, which records the speed of reaction as measured by variations in the pH values of soil suspensions to which different amounts of NaOH and $Ba(OH)_2$ were added. The soil was first treated with 0.05 N HCl exaustively to bring it to the fully unsaturated condition (acidoid state).

It will be seen from table 1 that the speed of reaction depends on the strength of the base used; and equilibrium could be reached fairly quickly by using excess of alkali. It seems, therefore, that the best method of bringing the soil to the saturation or neutralization point would be to add excess of alkali and then remove the excess by leaching or precipitating as an insoluble salt. This point is recognized in the author's method of finding saturation capacity of soils for bases (6)· where $Ba(OH)_2$ solution is added and excess removed by titration to phenolphthalein with 0.1 N H_2SO_4. The following method was used to bring about a similar state of affairs in the reaction with ammonia.

Ten grams of soil is stirred with about 50 cc. of water. Enough 0.2N HCl to break up all the carbonates is added gradually and the mixture set aside with frequent shaking till no more bubbles of CO_2 are formed. The soil is then transferred to a filter paper and washed with 0.05N HCl till the filtrate shows no reaction for Ca ions; this is followed by leaching with water till the soil is free from Cl ions. It is then washed with a little alcohol and air dried. The final washing with alcohol makes the soil loose and friable as well as easily detachable from the filter paper.

For washing purposes, a Buchner funnel is generally convenient; though a better method is to use filtering apparatus described by the writer elsewhere (3), with the modification that the cylinder portion need only be 2 or 3 inches long and provided with a lip.

After being air dried the soil is transferred to a flat dish. If a hardened filter paper, like

the Watman 50, is used the soil comes off quantitatively, with the help of a spatula and camel's hair brush. The dish containing the soil is kept over normal ammonia in a vacuum desiccator for two days. It is then transferred to another desiccator and kept over 90 per cent H_2SO_4 under vacuum for 48 hours. The ammonia retained by the soil is then determined by distillation with lime in the usual way.

DISCUSSION OF RESULTS

The results of ammonia reaction with 45 soils are recorded in table 2, which also gives the clay contents and pH values of the original and of the ammonia saturated soils.

It is seen that except in the case of soils free from carbonates (acid soils) or those containing Na_2CO_3 (alkali soils), the pH value of the original soil is approximately the same as after acid treatment and ammonia saturation. The reason is not hard to find. In almost all cases where the agreement is apparent, the soil contains excess of $CaCO_3$ and has reached a state of saturation with respect to Ca, in the sense that any excess of the Ca added to it will either be converted into carbonate or washed down if the soil is subjected to leaching. In other words it represents a limit beyond which any base, if present, will be easily hydrolyzed and removed by leaching.

TABLE 1

Speed of reaction between soil and $Ba(OH)_2$ and NaOH

(10 gm. of soil kept with 100 cc. alkali solution)

M.EQ. OF ALKALI	pH AFTER							
	1 day	2 days	4 days	6 days	7 days	10 days	11 days	22 days
(NaOH)								
2	5.98	5.30	...	5.06	...	4.86	...	4.63
5	9.18	7.20	...	6.85	...	6.71	...	6.45
10	11.74	11.60	...	11.60	...	11.57	...	11.57
$Ba(OH)_2$								
2	4.64	4.23	4.00	...	3.83	...	3.75	3.65
5	7.85	7.00	6.49	...	6.00	...	5.90	5.74
10	11.00	10.80	10.66	...	10.65	...	10.60	10.57

The mechanism of ammonia reaction is similar: the soil is allowed to take up an excess and the easily hydrolyzable portion is removed under vacuum over H_2SO_4. Thus the soil come to equilibrium at a pH value which is not far different from that found under natural condition in the presence of excess $CaCO_3$. However in the case of soils having no $CaCO_3$, or containing alkali salts, the pH value in the natural state may be too low or too high as compared to that obtained after ammonia saturation.

It must be pointed out, however, that the difference between hydrolyzable and non-hydrolyzable bases is only of degree; and there is no reason to suppose that almost all the bases will not be removed, provided the leaching action is

TABLE 2

Interaction between ammonia and various soils

SOIL	CLAY (0.002 MM.)	NH_3 ABSORBED		NH_3 SATURATED SOIL	ORIGINAL SOIL
		Per 100 gm. soil	Per gm. clay		
	per cent	*m eq.*	*m.eq.*	*pH*	*pH*
1	11.3	5.3	0.469	8.21	8.45
3	62.2	63.1	1.015	8.01	7.64
4	15.2	8.2	0.539	8.40	8.55
5	12.3	7.32	0.595	8.44	8.77
6	28.4	11.0	0.387	8.14	5.29
7	21.8	7.1	0.326	8.88	9.58
8	25.2	18.7	0.742	8.36	8.41
9	21.6	8.1	0.375	7.74	5.76
10	35.6	25.1	0.705	8.28	8.71
11	32.8	23.7	0.722	8.55	8.77
12	7.2	4.6	0.639	7.83	5.83
15	22.4	14.3	0.638	7.97	7.71
16	8.7	4.9	0.563	8.44	8.74
17	14.1	8.2	0.581	8.32	8.20
18	22.6	11.7	0.518	8.19	5.79
19	42.3	24.05	0.569	8.46	8.40
20	8.1	4.4	0.543	8.12	5.64
21	13.5	12.9	0.955	8.37	8.25
22	15.1	12.7	0.841	8.29	6.85
23	11.3	11.1	0.982	8.24	7.41
24	9.7	8.8	0.907	8.51	8.59
25	4.0	2.1	0.525	8.65	7.40
26	22.5	5.7	0.253	8.41	8.11
27	53.2	51.3	0.964	8.52	9.03
29	63.0	49.1	0.779	8.54	8.05
30	54.1	57.5	1.063	8.59	8.45
31	22.8	17.1	0.75	8.26	8.01
32	64.6	43.9	0.68	7.88	5.05
33	2.6	2.4	0.923	8.57	10.18
34	11.3	7.0	0.620	8.39	7.63
35	18.3	11.6	0.634	8.50	7.98
36	11.7	6.85	0.585	8.51	8.46
37	4.1	7.60	1.853	8.38	5.72
38	52.9	47.3	0.894	8.47	8.29
39	8.4	11.9	1.417	8.49	9.11
40	13.1	11.0	0.84	8.54	7.65
41	53.4	51.7	0.968	8.63	8.74
42	53.4	56.3	1.054	8.90	9.00
43	21.6	15.3	0.708	8.62	8.41
44	8.4	6.5	0.774	8.73	8.54
45	11.1	5.8	0.522	8.59	7.45
46	56.4	51.2	0.908	8.51	7.63
48	19.8	4.7	0.237	8.57	8.55
49	27.3	17.0	0.623	8.15	6.33
50	17.7	9.6	0.542	8.51	8.54

sufficiently prolonged. Another point worthy of note in this connection is that the removal of bases by simple leaching must depend entirely on the mutual reaction of the two bodies, i.e. only if the pH value of water is lower than that of the suspension of soil in the water, will bases be removed by leaching with that water.

It is interesting to note that the pH value at the saturation point for most soils is somewhere about 8.5, but it is appreciably lower in the case of all humus and latarite soils. It would thus appear that acidity in such soils is not only produced by their peculiar geographical condition of being situated in humid regions, but the nature of acidoids in them is such that if converted into saloids, they would tend to hydrolyze at a lower pH value and thus become acid much more readily than others.

Alkali soils, on the other hand, generally show quite the opposite effect: their hydrolysis point is situated at a higher pH value, so that such soils by the very nature of things, would resist the removal of bases by leaching, and tend to remain alkaline.

It is customary to denote the base exchange capacity of a soil by the amount of total cations (including H) removable by neutral salt reaction. Such a procedure is not logically sound, since the total exchangeable H cannot be removed by neutral salt reaction because it can never raise the pH value of the soil above 7. Bases removed by neutral salt reaction can only be spoken of as exchangeable bases, and should not be confused with base exchange capacity: just as moisture content of a soil is not the same as its moisture holding capacity. The one represents a dynamic state, the other a static condition.

It is evident that ammonia reaction affords a simple method of determining base exchange capacity of soils, and, as such, could be used as a "single value" for characterizing them in any system of genetic classification. It has been generally recognized that colloids separated from different soils are not quite alike; though they show a similarity of behavior on the whole. It is hoped that the method outlined will prove of some value in bringing out such differences.

Although no attempt can be made at this stage to show that similarly constituted soils can be grouped together on the basis of base exchange capacity, it might be stated that, on the whole, dark colored soils show a higher value than red, lateritic, or humus soils. A somewhat similar conclusion has already been arrived at by Mattson from his extensive researches on the colloidal behavior of soils with varying silica sesquioxide ratios (1).

Reference has already been made to the fact that most of the soils in nature, in the presence of $CaCO_3$, tend to acquire a pH value of 8.5, which may be approximately taken as the limit beyond which bases, if combined, would be easily hydrolyzed. It is therefore conceivable that this stage of reaction might mark the maxima or minima of certain soil properties. The author's conclusion, arrived at elsewhere (2), that to produce maximum dispersion of

soil for mechanical analysis the suspension must be made alkaline to phenolphthalein, is thus rendered comprehensible.

SUMMARY

Soils react with bases very slowly, and equilibrium condition can be reached quickly only by adding excess of alkali and removing the excess by leaching or by precipitation as some insoluble salt.

A soil made completely unsaturated can combine with an amount of ammonia which may be considered equivalent to its base exchange capacity.

The pH value of soils containing excess $CaCO_3$, after dilute acid treatment and ammonia saturation is approximately the same as that of the soil in the natural condition.

REFERENCES

(1) MATTSON, SANTE 1926 The relation between the electrokinetic behavior and the base exchange capacity of soil colloids. *Jour. Amer. Soc. Agron.* 18: 456–470, 510–512.

(2) PURI, A. N. 1929 A new method of dispersing soils for mechanical analysis. *Mem. Dept. Agr. India* 10.

(3) PURI, A. N. 1929 A new percolating cylinder and some of its uses. *Agr. Jour. India* 24: 408.

(4) PURI, A. N. 1930 Studies in soil colloids: I. Base exchange and soil acidity. *Mem. Dept. Agr. India, Chem. Ser.* 11.

(5) PURI, A. N. 1930 Studies in soil colloids: IV. Methods of estimating soil colloids. *Ibid.* 11.

(6) PURI, A. N. 1930 Studies in soil colloids: V. Methods of determining saturation capacity and degree of saturation of soil colloids. *Ibid.* 11.

DETERMINATION OF THE PERCENTAGE BASE SATURATION OF SOILS AND ITS VALUE IN DIFFERENT SOILS AT DEFINITE pH VALUES[1]

W. H. PIERRE AND G. D. SCARSETH[2]

Alabama Agricultural Experiment Station

Received for publication May 5, 1930

Hissink (12) in his early work on base exchange in relation to soil reaction emphasized the importance and necessity of considering the degree to which the soil exchange complex is saturated with bases. He introduced into soil science the term "degree of saturation of soils." Numerically expressed, this is known as the percentage base saturation. Satisfactory procedures for the determination of base saturation have been slow in development, however, and the work on base exchange has dealt more with considerations of the exchangeable bases than of the exchangeable bases in relation to the total exchange capacity of soils, i.e. percentage base saturation. As far as soil reaction is concerned, the relation between the exchangeable bases or the exchangeable hydrogen and the total exchange capacity is far more important than is the knowledge of any one of these values considered alone.

Since soil acidity is due to the presence of exchangeable hydrogen, it might be supposed that a close correlation would exist between the hydrogen-ion concentration of soils and their percentage base saturation. Green (9) reports that he obtained a good correlation between the hydrogen-ion concentration of soils and their percentage base saturation. On the other hand, Joffe and McLean (13) state as a result of their work that the hydrogen-ion concentration of the water extract of soils adds little to the knowledge of the state of saturation. Very little data are presented by these investigators, and the fragmentary data obtained by other investigators aid little in clarifying this question. The recent development of more satisfactory methods, however, makes possible a more thorough study of the problem.

Not only is it desirable to study the relation between hydrogen concentration and percentage base saturation, but it is also of fundamental importance to study the relation between percentage base saturation of soils and plant growth. This paper will deal with the first of these problems. The latter problem has been given considerable study by one of the writers, and the results are presented in an accompanying paper (25).

[1] Published with the approval of the director of the Alabama Agricultural Experiment Station.

[2] Associate soil chemist and assistant soil chemist, respectively.

DETERMINING THE PERCENTAGE BASE SATURATION OF SOILS

Three values—those for the exchangeable hydrogen, the total exchangeable bases, and the total exchange capacity—may be concerned in determining the percentage base saturation of soils. Obviously, if any two of these values are known the third can be calculated as can also the value for percentage base saturation. Thus any one of the following procedures may be used for the determination of percentage base saturation of soils:

a. Determinations of total exchangeable bases and of exchangeable hydrogen.
b. Determination of total exchangeable bases and of total exchange capacity.
c. Determinations of exchangeable hydrogen and total exchange capacity.

Each of these procedures has been used by one or more investigators. No attempt will be made here to describe the various methods that have been used in studies of the base saturation of soils. Some interesting comparisons of methods have been made by Gericke (8), Harada (10), and Turner (29). It is obvious that the accuracy of the percentage base saturation data obtained will depend on the accuracy of the separate determinations used in obtaining the values.

Because of the difficulty, during the early investigations on base exchange, of determining the total exchange capacity or the saturation capacity of soils, procedure (a) was the one employed by Hissink (12) and Gedroiz (7) in their pioneer investigations. Later work by Kelly and Brown (15), Parker (19), and others showed, however, that the methods for determinations of exchangeable hydrogen as used by these early investigators were inaccurate. Moreover, it was found that determinations of the total exchangeable bases may be subject to a considerable error because of the solution of non-exchangeable bases by the neutral salt solution used for leaching (17, 23). No very satisfactory method was at first available, therefore, for determining the degree of saturation of soils.

More recent work has resulted in a better understanding of the base exchange reaction of soils, and with this better understanding have come better methods. Among the best known of these have been the method developed by Kelly and Brown (18) for determining the total exchange capacity and the methods of Parker (19) for determining the exchangeable hydrogen. Other methods for determining one or the other of the values required in calculating the degree of saturation of a soil have been proposed by various investigators (3, 10, 26, 29). Schollenberger's ammonium acetate method (26) of determining both exchangeable hydrogen and total exchange capacity is of special interest because of its relative simplicity. Instead of leaching the soil with barium acetate, as in Parker's method, he leached the soil with ammonium acetate and titrated the leachings in order to determine the exchangeable hydrogen content. Since the exchange complex was believed to be saturated with ammonium by this treatment, he determined the amount of ammonia absorbed as a measure of the total exchange capacity. In the experience of the writers, considerable diffi-

culty was found in getting good duplication in determining exchangeable hydrogen by this method, especially on sandy soils, where the amounts determined were small. For this reason Parker's method of determining exchangeable hydrogen seemed preferable. Schollenberger's method, however, makes possible the determination of exchangeable hydrogen and of total exchange capacity on the same soil sample and with few operations. The writers, therefore, studied the possibility of adapting Parker's barium acetate method of determining exchangeable hydrogen for the determination of the total exchange capacity somewhat on Schollenberger's principle. Such a method, if proved satisfactory, would be more simple and rapid than the method of Kelly and Brown (18), especially if the exchangeable hydrogen of the soil was also to be determined.

The method consisted of leaching 10 gm. of soil with 250 cc. of *N* barium acetate of pH 7.0 and titrating the leachate for exchangeable hydrogen. The soil was then leached with 250 cc. of neutral *N* NH_4Cl in order to replace the barium by ammonium, and the excess NH_4Cl was removed by washing with 95 per cent ethyl alcohol until chlorides no longer appeared in the leachate. The absorbed ammonia was then determined by transferring the soil into a kjeldahl flask, distilling with magnesium oxide, and titrating the distillate with 0.1 *N* H_2SO_4. In the following discussion this method will be referred to as the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method of determining total exchange capacity.

This method was compared with the $Ba(OH)_2 - NH_4Cl$ method of Kelly and Brown as slightly modified by Parker. Some of the results obtained have already been given by Parker (22). It was found that the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method almost invariably gave lower values than did the $Ba(OH)_2 - NH_4Cl$ method. The differences in the values obtained by the two methods, although not large numerically, were significant from a percentage standpoint and caused considerable difference in the values for percentage base saturation, especially with sandy soils of low total exchange capacities. This will be noted from a study of the data given in columns two and three of table 1 and also in columns three and four of table 2. In table 1 are also given the percentage base saturation values for the 15 Coastal Plain soils, calculated on the basis of these two methods of determining total exchange capacity. These soils had been limed six months previously with precipitated calcium carbonate at the rate of one ton to the acre in greenhouse pots. Since determinations of exchangeable hydrogen were made on both the limed and unlimed soil, this made possible calculations of the percentage base saturation at two uniform pH values. The results are given in the last four columns of table 1. It will be noted that significant differences were obtained in the percentage base saturation values, depending on which method was used in determining the total exchange capacity. This was especially true at the lower pH value.

It is evident that either the $Ba(OH)_2 - NH_4Cl$ method gives high values, the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method gives low values, or that there are discrepancies in both methods of determining total exchange capacity. Parker (22) has

suggested the former possibility on the basis that very drastic treatments of the soil with $Ba(OH)_2$ may further increase its total exchange capacity. On the other hand, Kelly and Brown (18) used a preliminary treatment with an alkaline solution because they believe that the affinity of the hydrogen ions for the exchange complex, particularly the organic exchange complex, is so great that complete reversibility is facilitated by the use of an alkaline solution.

In order to make a further study of these two methods of determining total exchange capacity, 10 of the soils used in this investigation were limed at two different rates, one calculated to bring them to neutrality and the other to above neutrality. After one year, when the calcium carbonate had come to equilibrium with the soil, determinations of total exchange capacity were made by the two methods on the treated and on the limed samples. The results are given in table 2. It was found that the total exchange capacity values as determined by the $Ba(OH)_2 - NH_4Cl$ method were practically the same for the limed and the unlimed samples of each soil. On the other hand, the values obtained by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method were greater with the limed soils than with the untreated soils. In the last two columns of the table are given the percentage differences in the total exchange capacity values obtained by the two methods. It will be noted that whereas the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method gave values considerably lower than did the $Ba(OH)_2 - NH_4Cl$ method on the unlimed soils, it gave values for the limed soil which were very similar to those obtained by the latter method.

These results can be explained on two different hypotheses. In the first place, it may mean that liming a soil, like treating it with an alkaline solution as in the $Ba(OH)_2 - NH_4Cl$ method, causes an increase in the total exchange capacity of the soil. On the other hand, the results might be explained on the basis of more complete replacement of hydrogen by the $Ba(OH)_2 - NH_4Cl$ than by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method. This would mean that the exchangeable hydrogen values obtained by the barium acetate method are low. The true exchangeable hydrogen values would then be represented by the sum of the hydrogen obtained by the barium acetate method and that which is further replaced by an alkaline solution as measured by the increase in total exchange capacity obtained by the $Ba(OH)_2 - NH_4Cl$ over that by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method. It is interesting to note in table 3 that the percentage base saturation values of the soils at pH 4.80 based on these two hypotheses are very similar. These values are quite different, however, from those obtained by using the results from the $Ba(OH)_2 - NH_4Cl$ method for total exchange capacity and the barium acetate method for exchangeable hydrogen. The writers believe that the first hypothesis is tenable and that the $Ba(C_2H_3O_2)_2 - NH_4Cl$ gives a better value for saturation capacity than does the $Ba(OH)_2 - NH_4Cl$ method. In the first place, the studies of Burgess (5), Magistad (19), and others lend considerable evidence to the belief that the total exchange capacity of a soil can be increased by treating it with an alkaline solution. Secondly, Parker (22) has shown that the barium acetate method

gives very similar values for exchangeable hydrogen as do two other methods based on different principles. Moreover, he found that the soils were approximately neutral in reaction after being leached with neutral barium acetate. The conception that the barium acetate method gives true values for exchangeable hydrogen is also believed to be in better harmony with the meaning of a saturated soil. Although there is some difference in opinion as to the meaning of the saturation or total exchange capacity of soils the writers are in agreement with the definition given by Bradfield (3) that saturation capacity refers to the limit to which a soil can be saturated with bases by means of neutral salts.

It may be tentatively assumed, therefore, that the higher total exchange capacity values obtained by the $Ba(OH)_2 - NH_4Cl$ method than by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method and the higher values obtained on limed than on unlimed soil by the latter method, are caused by a so-called "build-up" or increase in the total exchange capacity of the soil. For this reason the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method of determining total exchange capacity and the barium acetate method for exchangeable hydrogen were used in this investigation in calculating the percentage base saturation of the soils at the different pH values.

THE PERCENTAGE BASE SATURATION OF SOILS AT DEFINITE pH VALUES

In table 1, to which reference has already been made, are given the percentage base saturation values of 15 sandy Coastal Plain soils. Since limed and unlimed samples of these soils were available, it was possible to calculate these values at two different pH values. The calculated values for percentage base saturation at pH 5.55 and pH 6.0 are given in the ninth and in the last columns of this table. It will be seen that at pH 5.55 the percentage base saturation values ranged from 27.0 to 48.9, and at pH 6 0 beween 43.1 and 62.4. These data show that the soils of similar origin and degree of weathering may differ considerably in their degree of saturation at like pH values.

Soils formed under different climatic conditions and of various degrees of weathering had been studied by one of the writers in some accompanying studies of the relation between soil acidity and plant growth (24) and also in studies of the buffer capacity of soils. The soils investigated had each been brought to different degrees of acidity in pots in the greenhouse, by treatment with precipitated calcium carbonate or with acid. The treatments of the first 13 soils of table 4 are fully described in an accompanying paper (24). The last two soils of table 3 were originally very acid and therefore had received only lime treatments. In all cases the lime was added in the form of precipitated calcium carbonate. Both the lime and the acid were allowed to react with the soil in a moist condition for nine months before the studies of percentage base saturation were made. The hydrogen-ion concentration of the soils was determined by the "dialysis-colorimetric method" (25).

In table 4 are given the values for the percentage base saturation of the soils at five definite pH values. If, for example, the percentage base saturation

values at pH 4.80 are considered, it will be noted that there is a wide variation between soils in this regard. Whereas soils 674 and 675 were less than 10 per cent saturated with bases at pH 4.80, soils 670, 673, 686, and 641 were over 50 per cent saturated at this same pH value.

Similar differences in the percentage base saturation values of the various soils are obtained at the other pH values. As the soils approach neutrality,

TABLE 1

The percentage base saturation of soils based on two different methods of determining total exchange capacity

SOIL TYPE	TOTAL EXCHANGE CAPACITY		H-ION CONCENTRATION AND EXCHANGEABLE HYDROGEN OF SOILS				PERCENTAGE BASE SATURATION BASED ON DIFFERENT METHODS AT pH VALUES OF			
			Unlimed soil		Limed soil		pH 5.55		pH 6.0	
	By the $Ba(OH)_2 - NH_4Cl$ method	By the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method	pH	E. hydrogen	pH	E. hydrogen	$Ba(OH)_2 - NH_4Cl$ method	$Ba(C_2H_3O_2)_2 - NH_4Cl$ method	$Ba(OH)_2 - NH_4Cl$ method	$Ba(C_2H_3O_2)_2 - NH_4Cl$ method
	mgm. eq.	*mgm. eq.*		*mgm. eq.*		*mgm. eq.*	*per cent*	*per cent*	*per cent*	*per cent*
Norfolk sandy loam	4.24	3.54	5.45	2.40	6.00	1.33	48.0	37.7	68.6	62.4
Greenville loam	7.43	6.90	5.55	3.53	6.25	2.13	52.5	48.9	64.7	61.9
Orangeburg very fine sandy loam	7.25	5.60	5.55	3.40	6.15	2.20	53.0	39.3	65.5	55.3
Norfolk fine sandy loam	8.29	6.63	5.55	4.40	6.00	3.47	47.0	33.5	58.3	47.8
Orangeburg sandy loam	5.44	4.03	5.55	2.67	6.20	1.67	51.0	33.8	64.0	51.1
Susquehanna very fine sand	3.84	2.90	5.55	2.07	6.25	1.00	46.1	28.6	64.3	52.5
Norfolk sandy loam	4.05	3.02	5.30	2.47	6.25	1.47	45.3	27.0	57.1	43.1
Norfolk fine sand	3.19	2.50	5.05	2.20	6.20	1.20	44.4	28.9	57.0	45.1
Norfolk loam	5.19	3.88	4.95	2.94	5.65	2.13	56.7	42.1	....	....
Greenville sand	1.62	1.71	5.45	1.13	6.70	0.20	35.0	38.0	56.0	58.1
Greenville pebbly loam	2.85	2.70	5.40	1.60	6.60	0.73	47.6	44.5	59.3	57.0
Norfolk sandy loam	3.71	3.00	5.55	1.87	6.35	0.87	49.6	37.7	65.0	56.8
Norfolk sandy loam	2.85	2.52	5.40	1.60	6.30	0.80	48.4	41.8	62.8	57.9
Greenville sand	1.71	1.46	5.20	1.13	6.80	0.33	44.0	34.2	57.5	50.2
Orangeburg fine sand	1.86	1.43	5.40	0.87	6.85	0.20	56.0	42.7	68.0	58.5

the differences in the percentage base saturation values between the various soils become less. This should be expected if soils are completely saturated with bases at pH 7.0. It will also be noted that as the soils approach neutrality they approach complete saturation. These results are typical of results obtained on other soils. With most soils it has been found that complete saturation, as determined by the methods used, is reached at pH 7.0, which fact

TABLE 2

The total exchange capacity of untreated and of limed soils as determined by the Ba $(OH)_2$—NH_4Cl and the $Ba(C_2H_3O_2)_2$-NH_4Cl methods

SOIL NUMBER	UNTREATED SOIL			SOIL LIMED TO ABOUT NEUTRALITY		SOIL LIMED TO ABOVE NEUTRALITY			PERCENTAGE DIFFERENCE IN TOTAL EXCHANGE CAPACITY BY THE TWO METHODS	
		Total exchange capacity			Total exchange capacity		Total exchange capacity			
	pH value	$Ba(OH)_2$ — NH_4Cl method	$Ba(C_2H_3O_2)_2$ — NH_4Cl — method	pH value	$Ba(C_2H_3O_2)_2$ — NH_4Cl — method	pH value	$Ba(OH)_2$ — NH_4Cl method	$Ba(C_2H_3O_2)_2$ — NH_4Cl — method	Untreated soil	Soil limed to above neutrality
		mgm. eq	*mgm. eq*		*mgm. eq.*		*mgm. eq.*	*mgm. eq*	*per cent*	*per cent*
670	5.20	29.37	26.33	6.80	27.43	7.43	29.37	28.00	10.3	4.7
671	4.50	8.88	7.76	6.68	8.40	7.40	9.09	8.78	12.6	3.4
672	5.80	7.29	5.50	6.78	6.25	7.43	7.24	6.57	24.6	9.2
673	6.43	9.47	9.41	7.22	9.40	7.45	9.43	9.12	0.6	3.2
674	6.00	5.57	4.85	6.80	5.18	7.45	5.60	5.28	12.9	5.7
675	5.75	2.35	1.83	7.00	2.05	7.52	2.29	2.25	22.1	1.7
676	5.60	14.54	13.28	6.80	13.21	7.38	14.80	13.57	8.7	8.3
678	5.53	4.35	4.00	7.38	.....	7.43	4.64	4.28	8.0	7.8
679	5.80	10.68	9.79	6.93	10.43	7.38	10.71	10.65	8.3	0.6
680	5.38	8.26	6.97	7.00	7.65	7.45	8.19	7.75	15.6	5.4
Average...	5.60	10.08	8.97	6.94	.....	7.43	10.13	9.63	12.3	4.4

TABLE 3

The percentage base saturation of soils at pH 4.80 as determined by different methods

SOIL NUMBER	METHOD 1	METHOD 2	METHOD 3
670	57	52	62
671	32	28	41
672	10	8	32
673	77	76	77
674	6	5	19
675	9	7	28
676	32	29	37
678	20	18	28
679	43	40	45
680	18	15	31

Method 1 is based on the total exchange capacity determined by the $Ba(C_2H_3O_2)_2$—NH_4Cl method and on the exchangeable hydrogen determined by the barium acetate method.

Method 2 is based on the total exchange capacity determined by the $Ba(OH)_2$ —NH_4Cl method, and the exchangeable hydrogen obtained by the barium acetate method plus the hydrogen measured by the difference between the values for total exchange capacity obtained by the $Ba(OH)_2$ — NH_4Cl and the $Ba(C_2H_3O_2)_2$ — NH_4Cl methods.

Method 3 is based on the total exchange capacity determined by the $Ba(OH)_2$ — NH_4Cl method and the exchangeable hydrogen determined by the barium acetate method.

indicates that the exchange complex saturated with calcium as the principle exchange cation does not undergo much hydrolysis.

In general, the soils from the Coastal Plain and Piedmont Plateau Provinces are lower in percentage base saturation than the other soils which are younger and less highly weathered. Three apparent exceptions, however, were obtained. These were the two Susquehanna soils and the Octibbeha clay. All these soils had a higher degree of saturation at given pH values than the other soils of the Coastal Plain, and two of these had just as high values as most of the other soils studied. It is significant, however, that all three of these soils are from

TABLE 4

The percentage base saturation of soils of different origins at like pH values

SOIL NUMBER	SOIL TYPE	LOCATION OF SOIL	TOTAL EXCHANGE CAPACITY	PERCENTAGE BASE SATURATION AT pH VALUES OF				
				4.80	5.00	5.50	6.00	6.50
			mgm. eq.					
670	Grundy silt loam	Ill.	26.33	57	60	69	80	91
671	Cory silt loam	Ill.	7.76	32	38	56	74	88
672	Cecil sandy loam	S. C.	5.50	10	20	36	53	69
673	Delta light silt loam	Miss.	9.41	77	80	86	92	94
674	Cecil clay loam	Ala.	4.85	6	23	41	58	74
675	Norfolk sandy loam	Ala.	1.83	9	16	32	44	60
676	Colby silt loam	Wis.	13.28	32	37	54	66	78
678	Cecil clay	Ala.	4.00	20	39	52	68	85
679	Miami silt loam	Wis.	9.79	43	50	63	72	82
680	Greenville fine sandy loam	Ala.	6.97	18	21	34	54	71
683	Susquehanna fine sandy loam	Ala.	4.05	28	41	50	70	....
684	Norfolk sandy loam	Ala.	3.00	20	31	40	59	....
685	Greenville sandy loam	Ala.	2.33	....	43	59	70	84
686	Oktibbeha clay	Ala.	19.36	52	66	78	82	86
641	Susquehanna clay	Ala.	34.25	55	70	79	85	94
Average of all soils			10.18	33	43	55	68	81

the Coastal Plain Black Belt, and that they are less highly weathered than the other soils of the Coastal Plain or Piedmont Plateau provinces.

The values for percentage bases saturation at pH 4.80 for soils 672, 674, and 675 seem low. They are what might be expected, however, when it is considered that the pH values of the desaturated colloids from these soils were found by Baver and Scarseth (2) to be between 4.40 and 4.75, whereas those of the colloids from the soils giving higher degrees of saturation were pH 4.0 or less. On the other hand, the base saturation values for some of the other soils at low pH values seem high. It is difficult to understand, for example, how soil 673 can be 77 per cent saturated with bases at pH 4.80. Some evidence for the belief that this value may be high has been obtained by Baver and Scarseth

(2) who determined the percentage base saturation from the titration curve of the electrodialyzed colloid from this soil. They obtained by this method considerably lower degrees of saturation than are here reported. When the exchangeable bases in this soil at pH 4.75, determined by electrodialysis, were used for the calculation of percentage base saturation, however, very similar values were obtained to those reported in the present study. As a further check on this soil, the exchangeable bases of the treated soil having a pH value of 4.75 and of the soil having a pH value of 5.80 were determined by Kappen's method (15). The value obtained at pH 4.75 was 8.80 mgm. equivalents and at pH 5.80, 10.50 mgm. equivalents. The exchangeable hydrogen values for these two samples were 2.80 and 0.90 mgm. equivalents, respectively. It appears that the values for exchangeable bases are higher than those obtained by difference in the present study. This is probably due to a solubility of non-exchangeable bases by the 0.1 *N* acid used in Kappen's method. It is significant, however, that the percentage base saturation values based on Kappen's method for exchangeable bases and those based on the barium acetate method for exchangeable hydrogen agree closely. A further study of this soil is being made, but it appears from the evidence at hand that although the values obtained may be slightly high this soil has a high percentage base saturation at low pH values. Possible explanations for the difference in the percentage base saturation of different soils at like pH values will be considered in the next section.

It is interesting to compare briefly the data on percentage base saturation obtained in this study with some of those obtained by other investigators who have used different methods. Mention will not be made here of data based on Hissink's original method, for Harada (10), Turner (29), and others have shown that his method gives low values for percentage base saturation.

Page and Williams (20) obtained base saturation values of 7.9 per cent at pH 4.04 and 29.0 per cent at pH 4.48 for soils from the Rothamsted Grass Plats.

Joffe and McLean (14) reported the percentage base saturation value for six soils and their subsoils of the Chenango series. Three of the soils had pH values of 5.0, and the percentage base saturation values were 43.70, 49.30, and 36.92. The subsoils showed a higher degree of saturation than the surface soils of similar pH values.

Conrey and Schollenberger (6), using the method previously referred to, determined the percentage base saturation of various horizons of a Clermont silt loam. They also obtained some evidence that the deeper subsoil is more highly saturated than the surface soil at the same hydrogen ion concentration. The surface soil at pH 5.92 was 48 per cent saturated, whereas the soil sample at 36 to 48 inches at the same pH value was found to be 81 per cent saturated with bases.

Harada (10), working with Japanese soils, reported percentage base saturation values for 16 soils whose pH values ranged from 4.48 to 6.43. The percentage base saturation values ranged from 13.6 with a soil of pH 5.94 to 77.2

with a soil of pH 6.0. Although he states that the degree of saturation can be correlated with the pH values, it is evident from his results that soils of the same pH values may have very different degrees of saturation.

Although close agreement cannot be expected between the results obtained by these various investigators and those reported in this study because of differences in soils and in the methods used, nevertheless, the data tend to substantiate the fact that soils of the same pH values may be very different in regard to percentage base saturation.

The differences obtained in the percentage base saturation of various soils at like pH values are of considerable practical as well as theoretical importance. These results show very definitely that the hydrogen ion concentration of soils does not truly represent the condition of soils with respect to acidity. In an accompanying paper (24) it is shown that within certain limits the growth of acid-sensitive crops on acid soils is much more closely correlated with the percentage base saturation than it is with the hydrogen-ion concentration of the soils. Likewise, it may be true that the biological activities of soils are more dependent on the degree of saturation of the soils than on their hydrogen-ion concentration.

FACTORS AFFECTING THE PERCENTAGE BASE SATURATION OF SOILS AT DEFINITE pH VALUES

In explaining the fact that soils differ greatly in their percentage base saturation at given pH values, the following factors should be considered:

Presence of soluble acids
Nature of the bases in the exchange complex
Nature of the exchange complex as might be revealed by
- a. Organic matter content of the soil
- b. Silica-sesquioxide ratio of soil colloid
- c. Total exchange capacity of soil colloid

Strength or avidity of the soil acids.

It might be expected that the presence of soluble acids in a soil would cause a relatively high degree of saturation at given pH values. Although most of the soils used in this study received acid treatments they were thoroughly leached to remove the soluble acids, and cropped to corn before they were studied. Some evidence for the effect of soluble acids was noted, however, with some other sandy soils which had been heavily fertilized with ammonium sulfate in the greenhouse and not subjected to leaching. Under ordinary conditions, however, the presence of soluble acids is not an important factor and cannot explain the major differences between soils obtained in this study.

A study was made of the nature of the bases in the exchange complex of some of the soil colloids. Since soils containing in the exchange complex monovalent bases, such as sodium, hydrolyze to a greater extent than do those containing divalent bases, such as calcium, it might be expected that the relative proportion of the various bases in the exchange complex would have some influence

on the percentage base saturation of soils at like pH values. It was found that calcium and magnesium constituted the greater portion of the exchangeable bases of all the colloids, and no correlation could be established between the proportion of monovalent to divalent bases and the degree of saturation at like pH values. For example, soil colloids 673 and 683 had higher proportions of monovalent to divalent bases than most of the other colloids and yet their percentage base saturation at given pH values is high. The data obtained, however, are not conclusive, for the colloids were of different degrees of saturation. It is very probable that if a soil contains a large proportion of exchangeable sodium, it would, because of hydrolysis of the exchange complex, show a lower degree of base saturation at the various pH values than a similar soil containing a greater proportion of exchangeable calcium.

The fact that soils vary considerably in percentage base saturation at like pH values is not surprising, however, if it is considered that the nature of the exchange complex of various soils may be quite different, and that the acid in the exchange complex of one soil may differ from that in the exchange complex of another soil. It seemed of interest, therefore, to study briefly some soil properties which might be correlated with the nature of the soil acids present and consequently with the percentage base saturation of soils at like pH values.

Since the organic matter of soils has base exchange properties, it might be argued that the percentage base saturation of the organic exchange complex is different from that of the inorganic exchange complex at like pH values. Soils with a high content of organic matter might, therefore, be expected to give different percentage base saturation values at a certain hydrogen-ion concentration than soils which have a very low content of organic matter. A study of the organic matter content of some of the soils investigated, however, failed to show any relationship between the percentage of organic matter and the percentage base saturation of the soils at like pH values. This will be noted by referring to table 1 of the accompanying paper (24). It is recognized, however, that a determination of total organic matter probably gives little indication of the organic matter that is active in base exchange or of the nature of the organic acids present.

Parker and Pate (23) and Anderson and Mattson (1) have shown that the silica-sesquioxide ratio of soil colloids bears a relation to their base exchange properties. That being true, it was thought that some correlation might be found between the silica-sesquioxide ratio of the soil colloids and the percentage base saturation of the soils at like pH values. Eleven soil colloids, which had been extracted by means of the supercentrifuge, were analyzed and their silica-sesquioxide ratios calculated. The results obtained are presented in table 5. A study of the ratios obtained and of the percentage base saturation values at like hydrogen-ion concentrations shows that in general soils of a high ratio seem to have higher degrees of saturation than soils with colloids of low silica-sesquioxide ratio. For example, the five soils which have the highest silica-sesquioxide ratio are also the five soils which have the highest degree of satura-

tion at pH 4.80. It is also significant that these five soils are less highly weathered than the other six soils showing a lower degree of saturation and a lower silica-sesquioxide ratio.

TABLE 5

The silica-sesquioxide ratio and total exchange capacity of extracted soil colloids as related to the percentage base saturation of the soils at pH 4.80

SOIL NUMBER	SOIL TYPE	TOTAL SiO_2	TOTAL Al_2O_3	TOTAL Fe_2O_3	$\frac{\text{MOLS } SiO_2}{\text{MOLS } R_2O_3}$	TOTAL EXCHANGE CAPACITY	BASE SATURATION OF SOIL AT pH 4.80
		per cent	*per cent*	*per cent*		*mgm. eq.*	*per cent*
670	Grundy silt loam	41.32	17.85	8.75	2.99	..	57
671	Cory silt loam	49.31	21.54	7.23	3.20	22	32
672	Cecil sandy loam	39.79	32.25	8.99	1.78	14	10
673	Delta light silt loam	49.32	25.67	9.16	2.66	32	77
674	Cecil clay loam	34.35	29.13	12.55	1.57	13	6
675	Norfolk sandy loam	41.01	32.53	5.75	1.92	22	9
676	Colby silt loam	47.43	20.96	6.80	3.18	32	32
678	Cecil clay	36.00	33.45	12.38	1.48	15	20
679	Miami silt loam	52.90	18.02	7.15	3.97	30	43
680	Greenville fine sandy loam	39.83	33.95	8.86	1.62	20	18
683	Susquehanna fine sandy loam	39.55	26.69	7.44	2.01	33	28

TABLE 6

The strength or avidity of soil acids as related to the percentage base saturation of soils at pH 4.80

SOIL NUMBER	H-ION CONCENTRATION	EXCHANGEABLE HYDROGEN CONTENT	AVIDITY* OF EXCHANGEABLE HYDROGEN	PERCENTAGE BASE SATURATION AT pH 4.80
	pH	*mgm. eq.*		
670	4.80	11.15	22.4	57
671	4.85	5.25	18.0	32
672	4.80	4.95	12.8	10
673	4.80	2.20	20.0	77
674	4.75	4.75	13.8	6
675	4.75	1.70	16.5	9
676	4.80	9.10	20.5	32
679	4.93	5.15	18.0	43
680	4.80	5.70	14.4	18
683	4.85	2.57	17.0	28
684	4.80	2.47	14.6	20

* Avidity is the actual percentage of total H replaced by the base K when the amount of $KC_2H_3O_2$ used is chemically equivalent to the amount of exchangeable H.

The total exchange capacity of the soil colloids are also given in table 5, and it will be noted that there is also a general relationship between these values and the percentage base saturation of the soils at pH 4.80. A very definite

correlation, however, between the silica-sesquioxide ratio or the total exchange capacity of the soil colloids and the percentage base saturation of the soils at given pH values is not obtained and is probably not to be expected, for the former values can be considered only partially indicative of the chemical nature of the exchange complex.

Various investigators have shown that the acids found in different soils or even in the same soil may be quite different. Truog (28) in his early work on soil acidity found that the acids in different soils vary in strength or avidity, and he proposed a method for determining the strength or avidity of soil acids based on the competition of the soil acids and of acetic acid for the base, potassium. Later, Tidmore and Parker (27) and Parker (22), using Truog's principle, also obtained marked differences in the strength of acids found in different soils. Parker also obtained evidence that the acids in the exchangeable condition in the same soil may be quite different. These results might be taken to indicate that soil acids differ in their degree of dissociation. If that is true, the hydrogen-ion concentration of soils can be looked upon as dependent on the percentage base saturation and on the degree of dissociation of the soil acids. Soils whose acids have a high degree of dissociation would, therefore, have at any definite pH value a higher degree of saturation than soils whose acids have a lower avidity or are less dissociated.

Studies were made of the avidity of the acids of the soils used in this study. For this purpose the soil samples at a pH of about 4.80 were used. The procedure used was that described by Parker (21) except that the soil suspensions treated with potassium acetate were allowed to stand over night before filtering. The data obtained are given in table 6. The results show very definitely that the avidity of the soil acids from the different soils varies considerably. Since these soils were at about the same pH values, it is evident that the hydrogen-ion concentration of soils does not give an entirely accurate picture of the strength of soil acids. The avidity of the exchangeable hydrogen or rather of the acids in the exchange complex varies from 13.8 with soil 674 to 22.4 with soil 670. The data show that the soils of the Piedmont Plateau and Coastal Plains which have undergone the greatest degree of weathering have acids of lower strength or avidity than do the other less weathered soils. Moreover, it is significant that the soils with acids of high avidity have a higher degree of saturation at pH 4.80 than do soils with acids of low avidity. This shows that the difference between the percentage base saturation of different soils at like pH values can to some extent be explained by the fact that the acids of different soils at like pH values may differ considerably in strength or avidity. Further evidence for this conclusion is obtained from the data given by Tidmore and Parker (27, p. 333) in which they found that the Susquehanna clay subsoil had relatively much stronger acids as measured by Truog's avidity method and by the sugar invesion test than by the determination of hydrogen-ion concentration. It will be remembered that the percentage base saturation of the Susquehanna clay used in this study, which was obtained from the same area

as was the one they used, gave a high degree of saturation at given pH values. The recent results obtained by Baver and Scarseth (2) are also very significant. They found that the relative strength of the acids found in some of the colloids extracted from these soils, as measured by multiplying the amount of acids present in the desaturated soil colloid by the dissociation constant of the acids, were very similar to the values for the relative strength of the acids found in this study.

SUMMARY

A study was made of the determination of the percentage base saturation of soils when based on the $Ba(OH)_2 - NH_4Cl$ and the $Ba(C_2H_2O_2)_2 - NH_4Cl$ methods of determining total exchange capacity. It was found that the $Ba(OH)_2 - NH_4Cl$ method gave values for total exchange capacity which averaged 17.9 per cent higher with a group of sandy soils and 12.3 higher with a group of heavier soils than did the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method. With the limed soils of the latter group the $Ba(OH)_2 - NH_4Cl$ method gave values which averaged only 4.4 per cent higher. These results are interpreted to mean that liming, like treating a soil with an alkaline solution, results in a "build-up" or the formation of additional exchange complex. For this reason the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method seems preferable in the determination of total exchange capacity. The percentage base saturation data given in this study were therefore calculated from the total exchange capacity values obtained by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method and the exchangeable hydrogen values obtained by the barium acetate method.

A study was made of the percentage base saturation of a number of soils of similar and of different origins at like pH values. It was found that soils of the same reaction may vary considerably in their percentage base saturation. In general, it was found that the highly weathered soils, such as those from the Piedmont Plateau and some from the Coastal Plain, have a lower degree of saturation at given pH values than less weathered soils, as those from the Coastal Plain Black Belt and the Glacial and Loessial Province.

No relation was found between the organic matter content of soils or the nature of the bases present in the exchange complex and the percentage base saturation of the soils at like pH values. The silica-sesquioxide ratio and the total exchange capacity of the colloids extracted from the soils showed a general, although imperfect, correlation with the percentage base saturation values of the soils at like pH values.

Determinations were made of the avidity of the soil acids in the different soils at similar pH values. It was found that the avidity or strength of the acids present was quite different for different soils. In general, highly weathered soils were found to have weaker acids than less weathered soils. A good correlation was obtained between the avidity of soil acids and the percentage base saturation of soils at pH 4.80.

REFERENCES

(1) Anderson, M. S., and Mattson, S. 1926 The properties of the colloidal soil material. U. S. Dept. Agr. Bul. 1452.

(2) Baver, L. D., and Scarseth, G. D. 1930 The nature of soil acidity as affected by the SiO_2-sesquioxide ratio. Paper presented at meeting of American Chemical Society, Atlanta. April, 1930.

(3) Bradfield, R. 1924 The importance of hydrogen-ion concentration in physico-chemical control studies of heavy soils. *Soil Sci.* 17: 411–422.

(4) Bray, R. H., and Filhite, F. M. 1929 The determination of total replaceable bases in soils. *Anal. Ed. Indus. and Engin. Chem.* 1: 144.

(5) Burgess, P. S. 1929 The so-called "build-up" and "break-down" of soil zeolites as influenced by reaction. Ariz. Agr. Exp. Sta. Tech. Bul. 28.

(6) Conrey, G. W., and Schollenberger, C. J. 1927 The effect of weathering on exchangeable bases as shown in the Clermont silt loam profile. *Proc. Internatl. Soc. Soil Sci.* 2: 212–229.

(7) Gedroiz, K. von 1924 Soils deficient in bases. Methods of determination of hydrogen-ions present in an absorbed condition. The requirement by soils of lime to neutralize unsaturation (trans. title). *Proc. Internatl. Soc. Soil Sci.* 2: 57 (abs.).

(8) Gericke, S. 1927 Comparative experiments on the methods for determining the saturation condition of the soil. *Zeitschr. Pflanzenerndhr. u. Dungung Bodenk.* A9: 20–24.

(9) Green, T. C. 1929 The relation of exchange calcium and magnesium to the reaction and inorganic content of soils. Ohio Agr. Exp. Sta. Bul. 431 (47th Ann. Rpt.)

(10) Harada, M. 1929 On the exchangeable bases in soils (trans. title). *Proc. Internatl. Soc. Soil Sci.* 4: 139 (abs.).

(11) Hissink, D. J. 1923 Methods for estimating absorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269–276.

(12) Hissink, D. J. 1925 Base exchange in soils. General views. *Trans. Faraday Soc.* 20: 551–566.

(13) Joffe, J. S., and McLean, H. C. 1926 Colloidal behavior of soils and soil fertility: II. The soil complex capable of base exchange and soil acidity. *Soil Sci.* 21: 181–195.

(14) Joffe, J. S., and McLean, H. C. 1927 Colloidal behavior of soils and soil fertility: III. Cation replacement and saturation of soil with Ca. *Soil Sci.* 23: 127–137.

(15) Kappen, H. 1928 Der Sattigungs zutand des Bodens und seine Bestimmung. *Fortschr. Landw.* 3: 109–114.

(16) Kelly, W. P. 1929 The determination of the base exchange capacity of soils and a brief discussion of the underlying principles. *Jour. Amer. Soc. Agron.* 21: 1021–1029.

(17) Kelly, W. P., and Brown, S. M. 1924 Replaceable bases in soils. U. of Calif. Tech. Paper 15.

(18) Kelly, W. P., and Brown, S. M. 1928 Base unsaturation in soils. *Proc. First Internatl. Cong. Soil Sci.* 2: 491–507.

(19) Magistad, O. C. 1929 The use of artificial zeolites in studying base exchange phenomena. *Jour. Amer. Soc. Agron.* 21: 1045–1056.

(20) Page, H. J., and Williams, W. 1924 Studies on base exchange in Rothamsted soils. General Discussion of Faraday Society, December, 1924.

(21) Parker, F. W. 1928 Methods for the determination of the amount and avidity of exchangeable hydrogen in soils. *Proc. First Internatl. Soc. Soil Sci.* 2: 164–174.

(22) Parker, F. W. 1929 The determination of exchangeable hydrogen in soils. *Jour. Amer. Soc. Agron.* 21: 1030–1039.

(23) Parker, F. W., and Pate, W. W. 1926. Base exchange in soil colloids and the availability of exchangeable calcium in different soils. *Jour. Amer. Soc. Agron.* 18: 470–482.

(24) Pierre, W. H. Hydrogen-ion concentration, percentage base saturation, and aluminum concentration in the soil solution as affecting plant growth on acid soils. *Soil Sci.* In press.

(25) Pierre, W. H., and Parker, F. W. 1927 The use of collodion sacks in obtaining clear soil extracts for the determination of the water soluble constituents. *Soil Sci.* 23: 13–32.

(26) Schollenberger, C. J. 1927 Exchangeable hydrogen and soil reaction. *Science* 65: 552–553.

(21) Tidmore, J. W., and Parker, F. W. 1924 Methods of studying the strength of soil acids. *Soil Sci.* 18: 331–338.

(28) Truog, E. 1916. A new apparatus for the determination of soil carbonates and new methods for the determination of soil acidity. *Jour. Indus. Eng. Chem.* 8: 341.

(29) Turner, P. E. 1928 An investigation of the method of Page and Williams for the determination of the saturation capacity of soils. *Jour. Agr. Sci.* 18: 257–265.

A MATHEMATICAL STUDY OF THE DECREASE OF CROP YIELDS

J. DUDLEY GREAVES

Utah Agricultural Experiment Station[1]

Received for publication May 3, 1930

The factors underlying and controlling the general processes of plant growth and nutrition are understood only to a very limited extent. It is known, however, that in any soil there are plant nutrients which are present in quantities smaller than required for optimum plant growth. The magnitude of the deficiency of any soil nutrient from the concentration required for optimum plant growth will constitute a limiting factor of plant growth in that soil. The most deficient nutrient will thus become a controlling factor of plant growth.

In order to obtain a first approximation of the influence of a deficient element on the crop yield we might make the following assumptions without serious error: (*a*) The rate of increase of crop yield with increase of the deficient element is proportional to the magnitude of the deficiency of the limiting nutrient from an optimum concentration; (*b*) the time rate of depletion of the deficient element, provided none is added from outside sources, is proportional to the product of the content of the deficient element in the soil and the crop yield. These two assumptions lead to the conclusion that the crop yield will diminish from year to year at a rate that will be dependent upon the physiological requirements of the crop for that element and upon the length of time plants needing that element have been grown upon the soil.

Using these assumptions, Greaves and Gardner (2) developed a theoretical formula showing the effect on crop yield when sulfur is the deficient element. Their formula was developed as follows:

Let y = crop yield per annum,
s = soil content of the deficient element,
s_0 = soil content of the deficient element for optimum growth of the crop,
k_1 and k_2 represent proportionality constants as yet undetermined,
t = time.

---

[1] Contribution from departments of physics and bacteriology. Publication authorized by director, July 11, 1929.

The author wishes to express his deep appreciation to Dr. Willard Gardner and to Dr. J. E. Greaves for their help and guidance, which has always been available and gladly given.

We may then express the assumptions in a mathematical form as follows:

$$(A) \qquad \frac{dy}{ds} = k_1 (s_0 - s)$$

$$(B) \qquad \frac{ds}{dt} = - k_2 (sy)$$

Integrating (A), we obtain

$$(C) \qquad y = k_1 \left(s_0 s - \frac{s^2}{2}\right) + k_3$$

Differentiating with respect to the time

$$(D) \qquad \frac{dy}{dt} = k_1 (s_0 - s) \frac{ds}{dt}$$

Eliminating $\frac{ds}{dt}$ by means of (B), equation (D) becomes

$$(E) \qquad \frac{dy}{dt} = - c (s_0 - s) sy$$

Where $c = +k_1 k_2$.

Equation (E) will integrate giving

$$ln \frac{y}{y_0} = - c \int (s_0 - s) sdt,$$

or written in exponential form

$$(F) \qquad y = y_0 e^{-c\int(s_0 - s) sdt}$$

If we were to assume the integration constant of equation (C), k_3, to be zero, the crop yield y will approach zero asymptotically. Equation (E) shows that the tangent to the curve of equation (F) will be zero when s is at an optimum value s_0. We can, therefore, assume without completing the integration that the curve of equation (F) will be of the form shown in figure 1. The slope of the curve will be determined by the crop grown.

In order to test further the validity of the foregoing assumptions, the author has taken the Rothamsted experimental data for those plots continually cropped without the addition of manure. Nitrogen in this case is probably the limiting plant nutrient.

The raw data were found to be very erratic, and in order to eliminate a part of this fluctuation, they were smoothed by taking an equally weighted moving average of 5 points. To do this, the first five crop yields were added together and then divided by 5; next the second to the sixth, inclusive, year crops were treated in the same manner. This was continued over the entire span of the experiment. In this manner it was possible to put the data in a rational form without markedly altering the general data curve.

Because of the difficulty involved in handling the exponential integral it was thought advisable as a first attempt to call[2] $-c\int(s_0 - s)\, sdt = -kt$. Thus, we assume $c(s_0 - s)s$ to be a function independent of the time. This assumption will put equation (F) in the form

$$(G) \qquad y = y_0 e^{-kt}$$

Taking the logarithm of both sides, equation (G) becomes

$$(H) \qquad z = z_0 - kt$$

Where

$$z = ln\, y$$

and

$$z_0 = ln\, y_0$$

Equation (H) being linear it should then be possible, if our assumptions are correct, to plot the logarithm of the yield against the time and get a straight line as a means of evaluating the constant k. This was done on all the available Rothamsted data with good confirmation of the theoretical values, as shown in figures 2–21, inclusive. It is to be noted, however, that all these curves show only a portion of the theoretical curve as plotted in figure 1. This is just as would be expected, for in each case the land selected for these experiments was in a partially rundown condition, having grown crops for many years under imperfect agricultural methods, also even many virgin soils are deficient in some plant nutrients.

Our work has been limited to the Rothamsted plots because of the long period for which accurate data are available for those plots. In this type of work data collected over short intervals of time may be misleading. For example, in figure 17 there is a marked decline during the years 1859–74 over that of the average trend of the data. It is again shown from 1914 to 1925. In this type of experimental work run under natural conditions there are environmental influences such as rainfall and temperature, which will alter greatly

[2] At the suggestion of Dr. Gardner the integration has been completed by eliminating s from equations (C) and (E), giving,

$$\frac{1}{s_0 - \sqrt{s_0^2 - \frac{2y}{k_1}}} + \frac{1}{2\, s_0}\, ln\, \frac{s_0 + \sqrt{s_0^2 - \frac{2y}{k_1}}}{s_0 - \sqrt{s_0^2 - \frac{2y}{k_1}}} = s_0 ct + \frac{1}{s_0}$$

the integration constant having been obtained by taking t equal to zero when s is at the optimum s_0. In effect, however, the approximation method here proposed gives a much simpler equation and, because of the somewhat erratic nature of the experimental data available, as revealed by the graphs, it has not been regarded as a profitable venture to attempt the evaluation of the three constants of this equation, although, it must be admitted, this would be another step toward a better representation of the yield-time relationship.

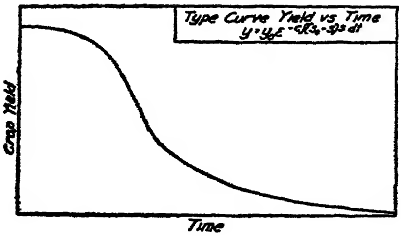


Fig. 1

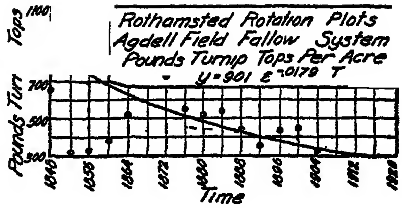


Fig. 2

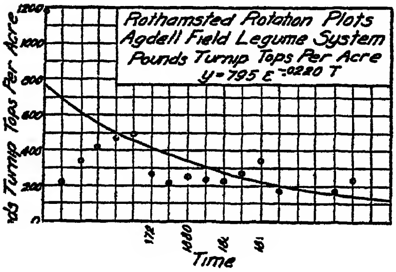


Fig. 3

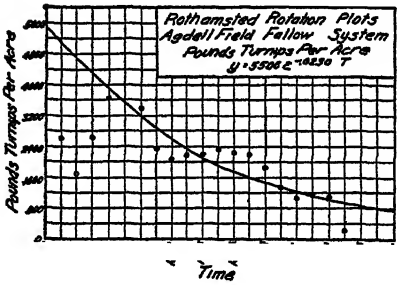


Fig. 4

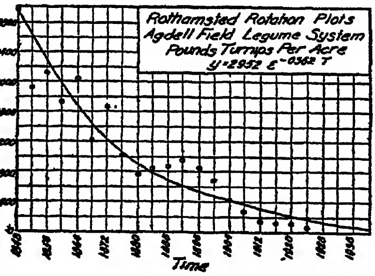


Fig. 5

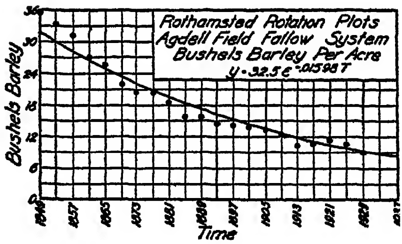


Fig. 6

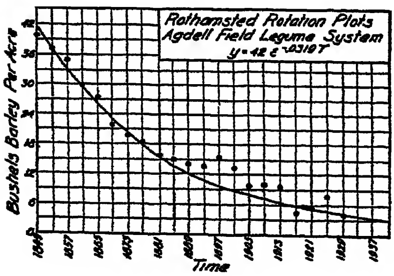


Fig. 7

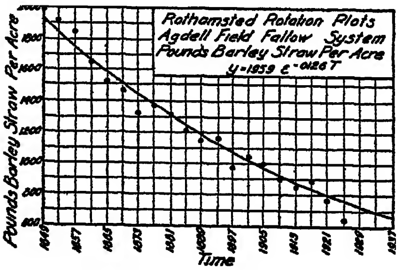


Fig. 8

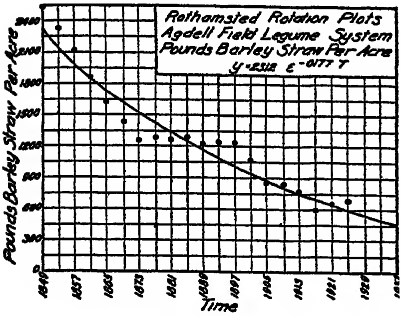


FIG 9

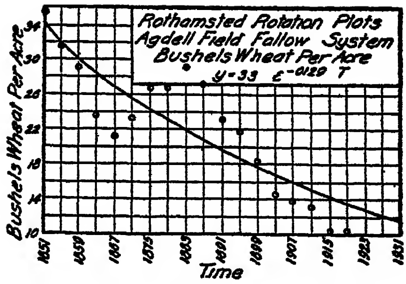


FIG. 10

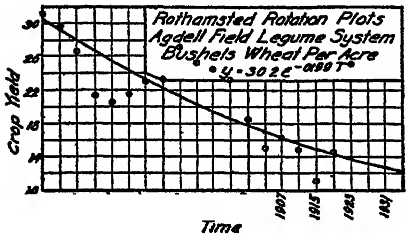


FIG. 11

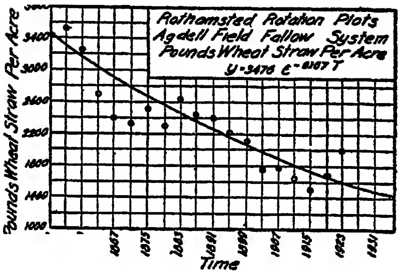


FIG. 12

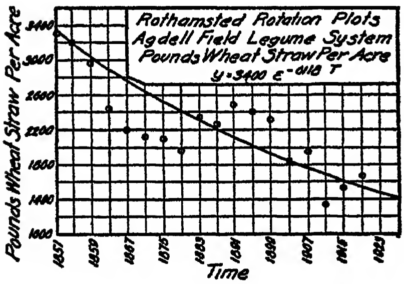


FIG. 13

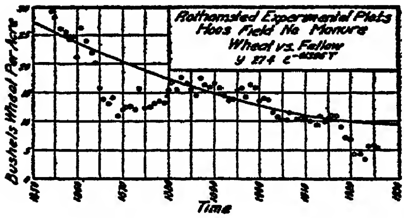


FIG. 14

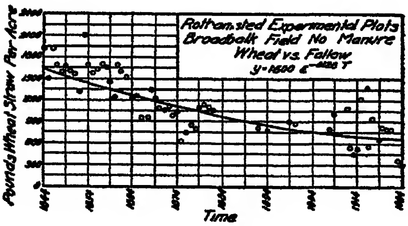


FIG. 15

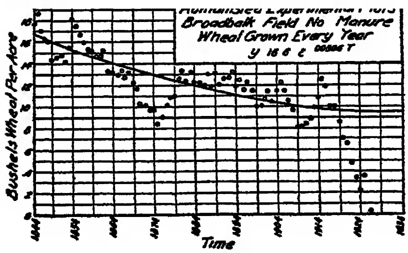


FIG. 16

the individual annual yields; yet when taken over a long period will tend to equalize themselves and so cause a reverting to the natural course.

The data taken from the plots of the Ohio and Illinois stations were considered, but because of the short time over which accurate data were available they have been omitted.

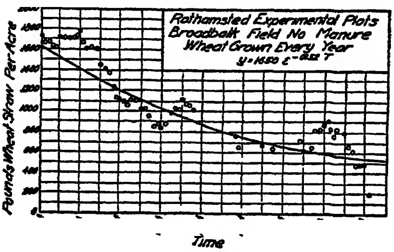


FIG. 17

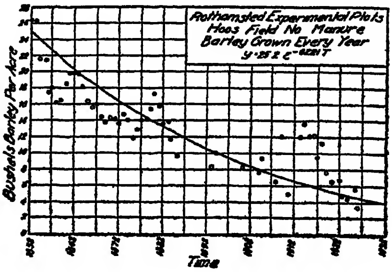


FIG. 18

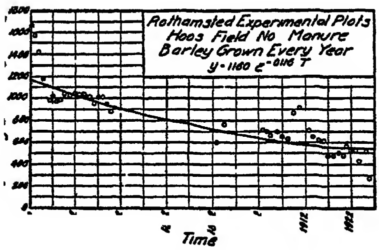


FIG. 19

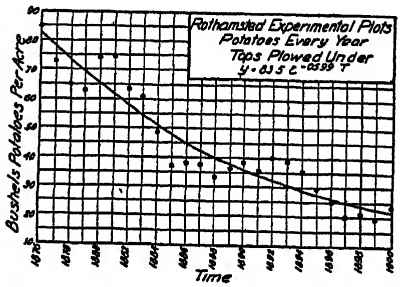


FIG. 20

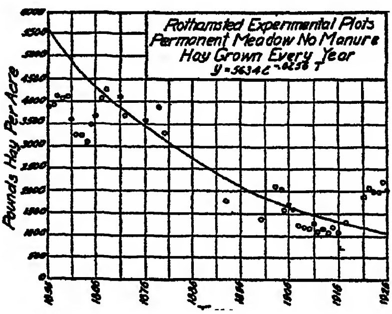


FIG. 21

We have defined our $k = c(s_0 - s)\, s$. Substituting this value in equation (E) we get

$$(I) \qquad \frac{dy}{dt} = -ky$$

It is thus seen that the rate of change of the crop yield with respect to the time is determined by the constant k. We may thus define k as the fractional rate of change of the crop yield from year to year.

It will be noted that in the rotation crop systems carried out on Agdell field the legume system constants are larger than the corresponding fallow system constants. This would tend to show that the use of a legume system of crop rotation will deplete the soil of its nutrients more rapidly than a corresponding fallow system of rotation, as earlier pointed out by Greaves (1).

TABLE 1

Summary of characteristic constants

CROP	PLACE	MAXIMUM	PROPORTIONALITY CONSTANTS
Turnip Tops	Agdell field, fallow system	901.0	0.018
Turnip Tops	Agdell field, legume system	795.0	0.022
Turnips	Agdell field, fallow system	5,506.0	0 023
Turnips	Agdell field, legume system	2,952.0	0.036
Barley Grain	Agdell field, fallow system	32.5	0.016
Barley Grain	Agdell field, legume system	42.0	0.032
Barley Straw	Agdell field, fallow system	1,959.0	0.013
Barley Straw	Agdell field, legume system	2,312.0	0.018
Wheat Grain	Agdell field, fallow system	33.0	0.013
Wheat Grain	Agdell field, legume system	30.2	0.020
Wheat Straw	Agdell field, fallow system	3,476.0	0.011
Wheat Straw	Agdell field, legume system	3,400.0	0.012
Wheat vs. fallow	Hoos field, no manure	27.4	0 014
Wheat vs. fallow	Broadbalk field, no manure	1,600.0	0.013
Wheat continually Grain	Broadbalk field	16.6	0.0081
Wheat continually Straw	Broadbalk field	16.5	0.015
Barley continually Grain	Hoos field	25.2	0.022
Barley continually Straw	Hoos field	1,160.0	0.012
Potatoes continually		83.5	0.060
Hay continually	Permanent meadow	5,634.0	0.026

In the Broadbalk field plots, in which wheat was grown continually, the fractional rate of decrease of straw from year to year was 0.0152, whereas in the rotation crop system on Agdell field it was 0.0107 for the fallow and 0.0118 for the leguminous. This would tend to show that the rate of decrease in crop yield with respect to the time is greater in the one crop system than in the rotation system. This same relationship holds on other similar comparisons, as shown in table 1. The continued growth of small grains does not tend to alter the physical conditions of the soil to the same extent as do crops such as hay and potatoes. This would tend, then, to explain the high constant values obtained for these two crops.

SUMMARY

An attempt was made to test further the equation for crop yield developed by Greaves and Gardner (2).

Their exponential integral was assumed to be a linear function of the time.

The formula was then tested on all available Rothamsted data with good confirmation.

This would tend to establish the two assumptions on which the formula was based:

The rate of increase of crop yield with increase of the deficient element is proportional to the magnitude of the deficiency of the limiting nutrient from an optimum concentration.

The time rate of depletion of a deficient element, provided none is added from outside sources, is proportional to the product of the soil's content of the deficient element and the crop yield.

REFERENCES

(1) GREAVES, J. E. 1918 Does crop rotation maintain the fertility of the soil? *Sci. Mo.* 6: 458–466.

(2) GREAVES, J. E., GARDNER, W. 1929 Is sulfur a limiting factor of crop production in some Utah soils? *Soil Sci.* 27: 445–457.

THE FUNGUS FLORA OF THE SOIL

H. L. JENSEN[1]

State Laboratory of Plant Culture, Lyngby, Denmark

Received for publication April 30, 1930

During the last few decades much research has been devoted to the study of the microscopic fungus flora of the soil, and it is at present a generally accepted view that all soils harbor a fairly definite flora of filamentous fungi, especially Mucoraceae and *Fungi imperfecti*. This fungus flora, which varies somewhat in its composition according to climatic and soil conditions, takes an active part in the microbial decomposition processes in the soil, under certain conditions apparently so energetically as to rival the importance of bacteria.

Most of these studies have been carried out on North American, a fair number also on European, soils. [For review of the very voluminous literature, see the works of Brierley (3) and Waksman (58).] In the Scandinavian countries, Hagem (18, 19) in Norway carried out a masterly study of soil Mucoraceae, and other valuable contributions have been furnished by Sopp (44) and Traaen (47). On the fungus flora of Danish soils the information is very scanty, Müller (36) was among the first botanists to point out that humus soils contain an abundance of fungal hyphae, and that these are of different types in peat and mold soils. Rostrup [see Müller et al. (37)] isolated on agar media from three uncultivated heath soils species of the genera *Oidium*, *Monilia*, *Penicillium*, *Dematium*, and *Hormodendron;* from five heath soils planted with pine trees Rostrup isolated the same species, and, further, *Mucor*, *Fusicolla*, *Fusidium*, *Trichoderma*, *Citromyces*, *Pachybasium*, *Verticillium*, *Stysanus*, and four sterile mycelia. Later Rostrup (43) isolated from various Danish soils: *Mucor ramannianus*, *Zygorhynchus vuilleminii*, *Absidia cylindrospora*, *Absidia rchidis*, and several species of *Penicillium*. Weis and Bondorff (66) isolated from acid forest soils *Mucor ramannianus*, four *Penicillia*, one *Citromyces*, and one *Sporotrichum*.

This paper represents an attempt to obtain some information about the typical groups of fungi in a series of Danish soils of widely varying character, especially cultivated soils, and about the distribution of fungi in relation to soil conditions, and their importance in certain biochemical soil processes.

[1] Now bacteriologist to The Linnean Society of New South Wales, Sydney.

The Qualitative and Quantitative Character of the Soil Fungus Flora

In this study, 100 different soils were included viz., 49 field soils, 6 garden soils, 18 forest soils, 12 moor and meadow soils, 5 heath soils, 5 marsh soils, and 5 other uncultivated mineral soils. Some samples were taken for this specific purpose, and the rest were soil samples forwarded to the laboratory for chemical analysis or lime requirement determination. The samples were examined as soon after arrival as circumstances would permit, and all numbers of microorganisms were calculated on the basis of fresh, moist soil. The following three methods were used: Microscopical examination (9), direct isolation of fungi (49), and plate countings.

MICROSCOPICAL EXAMINATIONS

The microscopical examinations were carried out with only 30 soils, but confirmed fully the statement of Conn (9): mineral soils were more or less poor in mycelium, though the acid soils seemed to harbor more than the neutral and alkaline ones. Of the humus soils the neutral and alkaline ones were not richer in mycelium than the mineral soils, but an abundance of mycelium was observed in the distinctly acid humus soils, especially those of peaty character. As already pointed out by Müller (36), mostly coarse, dark hyphae predominated in these soils; but it is noteworthy that a good deal of this mycelium was apparently represented by dead material—short fragments with frayed cell walls and apparently devoid of protoplasm; it might seem as if the abundance of mycelium is partly due to a defective power of these soils to decompose dead fungus mycelium. An exception was shown by a few samples of sphagnum-peat (samples 74 and 75, table 1), which in spite of their extremely acid reaction appeared very poor in mycelium. This is in agreement with the very small numbers of fungi shown by plate counts from these soils, and is probably connected with their poverty in mineral nutrients.

DIRECT ISOLATIONS

A number of experiments were made by the method suggested by Waksman (49) for distinguishing between spores and vegetative mycelium in the soil—inoculation of agar plates with lumps of soil and isolation of the fungi developing after 24 hours. The agar medium was that described in the following (p. 127), and 45 different soils were tested: 18 field soils, 15 forest soils, 4 moor soils, 4 heath soils, 2 marsh soils, and 2 uncultivated mineral soils. Fungus hyphae were found radiating from the soil in all cases after 24 hours' incubation at 25°C. The fungi were constantly Mucoraceae or Trichodermae, often both groups. They showed the following distribution within the different soil groups:

From *18 field soils* isolated:

	times
Mucor hiemalis (?)	4
Zygorhynchus spp	7
Absidia cylindrospora	5
Sterile Phycomycetous mycelium	7
Trichoderma spp	2

From *15 forest soils:*

	times
Mucor hiemalis (?)	1
Mucor circinelloides (?)	1
Absidia cylindrospora	2
Sterile Phycomycetous mycelium	3
Trichoderma spp	11

From *4 moor soils:*

	times
Mucor spinosus (?)	2
Sterile Phycomycetous mycelium	1
Trichoderma spp	4

From *4 heath soils:*

	times
Absidia orchidis	1
Trichoderma spp	4

From *2 marsh soils:*

	times
Zygorhynchus sp	1
Absidia cylindrospora	2

From *2 other soils:*

	times
Mucor hiemalis (?)	1
Zygorhynchus spp	2
Trichoderma sp	1

We see here that the fungi are largely the same as those obtained by Waksman (49) by direct isolation, and further that the soils fall into two well-defined groups; namely, field soils, marsh soils, and uncultivated mineral soils, which yield mostly Mucoraceae, especially *Zygorhynchus Absidia*, and a peculiar sterile Phycomycetous mycelium (*Cunninghamella elegans ?*); and forest, moor, and heath soils, of which the Trichodermae are characteristic. Since the soils of the latter group are, on an average, of a more acid reaction than those of the former, it might be imagined that this difference is due to the reaction, and that the Mucoraceae occur in an active state mostly in less acid, in neutral, or in alkaline soils, the Trichodermae in more acid soils. It is, however, not so certain that the fungi obtained by this method are the only ones present in the mycelial state, since they are all distinguished by their rapidly spreading growth and would easily depress more slowly growing forms. A point which greatly supports this view is that the slowly developing *Mucor ramannianus* occurs with so perfect constancy in all forest soils that one can hardly imagine it occurring only as spores; yet it has never been obtained by direct isolation. It is thus not easy to find an explanation for the difference between the two groups of soils; perhaps the fungi found by direct isolation are those which have the largest amount of mycelium in the soils in question. This would be in agreement with the fact that the Trichodermae, is as shown subsequently, display their greatest activity in distinctly acid soils.

PLATE COUNTINGS

Counts of fungi were carried out in all 100 solis according to the method outlined by Waksman (52), with some slight modifications.[2] The following

[2] The suggestions of Brierley et al. (4) for standardizing the technique in quantitative work with soil fungi unfortunately could not be used to advantage here, since the experimental part of this work was finished before the publication of Brierley's paper. The same is true of the method of McLennan (32) for distinguishing quantitatively between fungus spores and mycelium in the soil.

TABLE 1

Numbers of fungi, bacteria and actinomyces in soils of different character

NUMBER	CHARACTER OF SOIL	pH	FUNGI, PER GRAM	BACTERIA + ACTINOMYCES PER GRAM
			thousands	*millions*
	Field soils			
1	Sand	4.83	123.4	.....
2	Sand	5.15	135.4	.....
3	Sand	8.10	127.0	.....
4	Sand	7.22	92.4	13.4
5	Sand	6.59	218.0	12.0
6	Sand	6.91	166.6	21.6
7	Sand	7.03	41.4	15.7
8	Sand	6.98	79.0	17.6
9	Sand	6.91	87.2	22.5
10	Sand	7.16	108.6	15.8
11	Sand	6.78	145.6	14.9
12	Light loam	7.32	68.8	16.1
13	Light loam	7.15	100.4	17.6
14	Light loam	7.26	109.4	20.5
15	Loam	6.70	66.6	19.5
16	Loam	6.86	80.0	16.7
17	Loam	6.79	67.0	13.0
18	Light loam	6.30	63.0	.....
19	Light loam	6.36	80.8	.....
20	Heavy loam	6.22	107.9	15.3
21	Heavy loam	7.50	164.4	15.8
22	Heavy loam	7.52	78.0	16.2
23	Heavy loam	6.85	132.0	24.0
24	Sand	6.14	96.4	10.3
25	Sand	6.35	104.8	10.6
26	Sand	6.48	113.4	9.9
27	Sand	6.92	104.4	11.2
28	Sand	7.36	112.0	11.0
29	Sand	7.66	109.6	10.5
30	Sand	5.09	260.8	4.45
31	Sand	6.93	207.4	12.6
32	Sand	7.40	198.6	17.0
33	Light loam	6.72	86.4	.....
34	Light loam	7.86	154.6	.....
35	Light loam	5.92	131.4	7.40
36	Light loam	7.56	65.6	.....
37	Heavy loam	6.74	89.4	.....
38	Clay	4.96	98.0	1.75
39	Sand	5.59	87.2	.....
40	Humus	3.74	453.0	.....
41	Humus	7.08	174.0	25.9
42	Humus	6.09	188.0	19.3
43	Humus	7.20	124.0	7.80
44	Humus	3.76	182.8	0.40
45	Heavy clay	3.97	36.7	1.03
46	Heavy loam	8.35	75.6	11.1
47	Heavy loam	7.02	145.0	3.53
48	Heavy loam	7.23	146.6	10.9
49	Light loam	5.56	257.4	11.5
	Garden soils			
50	Light loam	7.15	252.4	48.3
51	Light loam	7.27	154.8	.....
52	Light loam	7.18	154.0	.....
53	Humas	7.22	176.0	.....
54	Light loam	7.38	460.0	102.4
55	Light loam	7.78	173.2	25.7
	Forest soils			
56	Loam	7.75	166.0	22.3
57	Sand	5.66	40.4	.....
58	Sand	4.83	64.0	.....
59	Sand	4.97	229.2	4.55
60	Sand	5.12	78.0	0.83
61	Humus ("Mould")	6.32	46.0	5.92
62	Humus ("Mould")	5.94	45.6	.....
63	Humus ("Mould")	6.06	52.4	6.40
64	Humus ("Mould")	4.12	165.0	0.67
65	Humus ("Mould")	3.34	108.6	0.17
66	Humus (Peat)	4.32	82.0	0.61
67	Humus (Peat)	3.65	140.0	0.17
68	Humus (Peat)	4.01	174.0	.....
69	Humus (Peat)	4.53	56.0	.....
70	Clay	5.54	190.6	7.10
71	Sand	5.65	99.4	4.40
72	Humus	4.45	385.2	3.91
73	Peat	3.86	176.0	0.32
	Moor and meadow soils			
74	High moor	3.62	34.6	0.095
75	High moor	3.86	33.4	.....

TABLE 1—*Concluded*

NUMBER	CHARACTER OF SOIL	pH	FUNGI, PER GRAM	BACTERIA + ACTINOMYCES PER GRAM
			thousands	*millions*
76	High moor	5.14	62.0	4.64
77	High moor	4.58	220.6	1.06
78	High moor	4.24	75.0	0.58
79	Low moor	5.40	54.0	.....
80	Low moor	5.20	108.0	3.55
81	Low moor	5.64	124.0	3.36
82	Low moor	4.26	206.0	1.42
83	Low moor	7.61	121.2	39.3
84	Mud	4.17	274.6	1.10
85	Mud	6.90	68.6	23.3
	Heath soils			
86	Dry peat	4.40	40.4	.....
87	Dry peat	3.85	183.0	0.21
88	Dry peat	4.10	185.4	0.40
89	Dry peat	4.56	46.6	.....
90	Dry peat	4.39	127.4	1.23
	Salt marsh soils			
91	Very heavy clay	6.82	29.7	8.0
92	Very heavy clay	7.29	26.0	.....
93	Heavy clay	6.12	24.3	8.63
94	Heavy clay	6.29	31.0	.....
95	Very heavy clay	5.12	58.6	7.80
	Various uncultivated mineral soils			
96	Light loam	5.20	76.6	.....
97	Sand	7.55	100.0	21.8
98	Sand	8.04	68.0	.....
99	Sand	7.95	184.0	52.3
100	Light loam	8.08	149.2	57.6

medium was used: dextrose, 20 gm.; asparagin, 2 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.5 gm.; $FeCl_3$, 0.1 gm.; agar, 25 gm.; H_2O, 1,000 cc.; pH 3.8–4.0. This medium was found preferable to that used by Waksman, which, probably on account of its peptone content, was found to have a tendency to allow a too abundant development of the Mucoraceae and Trichodermae, so that the more slowly developing fungi may be entirely suppressed. The soil suspensions were prepared by shaking usually 10 gm. of soil with 200 cc. of sterile water for five minutes and transferring 1 cc. to 99 cc. of sterile water, whereby a solution of 1:2,000 is obtained. From this suspension 1-cc. portions were transferred to sterile Petri dishes, and a tube of melted agar, cooled to about 42°C., was added to each. Six or eight parallel plates were prepared and incubated for four or five days at 25°C. When the colonies were counted, a record was made of the relative abundance of the various types of fungi occurring on the plates, and representatives of the predominant forms were isolated. In 73 of the soils determinations of the numbers of bacteria and actinomycetes were also carried out. This also was done according to the method of Waksman (53), by plating on the following medium: dextrose, 2 gm.; casein, dissolved in 10 cc. 0.1 *N* NaOH, 0.2 gm.; K_2HPO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; $FeCl_3$, trace; agar, 15 gm.; pH 6.5–6.6. A dilution 1:10,000–50,000 was generally chosen for the acid soils and one of 1:100,000–200,000 for the neutral to alkaline soils; this gave in most cases 50 to 250 colonies

to each plate. The counting was carried out after 8 to 10 days' incubation at 25°C. Table 1 gives the results of these counts, together with general characterization of the soils and the pH values, measured by the quinhydrone electrode.

The composition of the fungus flora

The composition the fungus flora showed the following features:

The *Mucoraceae* are a typical group of soil fungi, represented in all the soils examined here. Most frequent were: *Mucor hiemalis*-like form; *Zygorhynchus* sp. (*Vuilleminii* ?); *Mucor ramannianus* which, as previously pointed out by Hagen (18), occurred with perfect constancy in all forest soils, generally accounting for the majority of the colonies here. It was further common in heath and moor soils and not rare in sandy field soils; it occurred in 17 sandy field soils, in 2 humus soils (nos. 42 and 43), and in only one loamy field soil. *Absidia cylindrospora* occurred with great frequency in soils of all types. A peculiar sterile Phytomycetous mycelium, which could not readily be identified with any previously described organism, was found more constantly than any other fungus; it occurred in all soils except 57, 62, 66, 67.[3] Besides these dominating forms several others were found, e.g. *Mucor spinosus* and *Absidia orchidis* in some heath and moor soils, and *Mucor racemosus*, *M. circinelloides* and some other non-identified forms. *Rizopus nigricans*, which according to Waksman (50) is one of the most common soil fungi in North America, was found only once (in 65), and *Rhizopus nodusus*, which has been found very commonly in Norway by Hagen (18), was not met with at all. The numbers of Mucor colonies on plates from field soils rarely amounted to more than 12 to 15 per cent of the total, whereas in the forest soils they often reached 25 to 40 per cent, and in the marsh soils they (especially *Absidia cylindrospora* and the sterile mycelium) were absolutely dominating, occupying 60 to 80 per cent of the total.

The next group of typical soil fungi is the genus *Trichoderma* which was represented in nearly all soils except the marsh soils, where it was found only in the strongly acid soil 95. Only once was a white strain found; all others were green, abundantly sporulating forms, mostly corresponding to the species *koningi* and *lignorum*, but so variable and with so many transition forms that a sharp distinction was impossible. A few less common forms were found, which probably represent separate "species;" one of these agreed well with Waksman's (48) strain III. The numbers of *Trichoderma* colonies on the plates were in most cases very small; only in a few cases did they cover 10 to 12 per cent of the total number, in one case (soil 64) even 30 per cent.

The genus *Penicillium* (including *Citromyces*) was, as might be expected, abundantly represented, and species of these were always the dominating forms in field soils, where they generally accounted for 50 to 60 per cent, sometimes 70 to 80 per cent, of the total number of colonies. In forest, heath, and moor soils, too, they were richly represented although often overshadowed by Mucoraceae and sterile mycelia. Marsh soils were poor in Penicillia; the only soil in which they were found at all was the alkaline marsh soil 92. About 40 strains were isolated, but could not be identified; one of them (*Penicillium* sp. I) was found more frequently and in higher numbers than any other; it seemed closely related to *Penicillium commune*. Also a *Citromyces* form (*glaber?*) was quite common.

The genus *Aspergillus* showed some interesting phenomena. These fungi occurred only sporadically in ordinary uncultivated and field soils. *Aspergillus ochraceus* (?) was found in

---

[3] On a later occasion a mycelium of this type, isolated from an English field soil, was found fructifying on Conn's glycerine-asparaginate-agar and appeared to be *Cunninghamella elegans* Lendner (28). The appearance of the mycelium from the Danish soils agreed very well with Lendner's description, but fructification was never seen to take place.

meadow soil 86, *Asp. fumigatus* in heath soil 89, and *Asp. globosus* (?) in uncultivated mineral soil 99. All garden soils, on the other hand, harbored *Asp. fumigatus*, and in the two greenhouse soils 53 and 54 this was the dominating form, occupying 35 and 70 per cent of the total numbers of colonies, respectively. The plates from the former soil had also a considerable number of colonies of *Asp. niger*. These findings are quite in agreement with the observations of Waksman (50) and Werkenthin (68), that Aspergilli are more common in soils from hot than from colder climates. Jensen (23) records only one species of Aspergillus from Alaska soil. Sopp (44) states that Aspergilli are rare in soils from Norway, and Traaen (47) records the occurrence but once in 70 mycological analyses of soils from the same country. This is not surprising in view of the fact that the Aspergilli are a group of somewhat thermophilic organisms, thriving excellently at 35 to 40°C., where the Penicillia are hardly able to live. The constant occurrence of *Aspergillus fumigatus* in garden soils of ordinary temperature may be due to its having been introduced with compost dung, where organic matter is usually decaying under aerobic conditions at a high temperature.

The next two groups of fungi seemed characteristic of cultivated soils. The first of these was the genus *Fusarium*, especially *Fusarium orthoceras*, which occurred in all field soils except 40, in the garden soils 51, 52, 55, and in the cultivated moor soils 76 and 79. The only uncultivated soils which harbored this species were the moor soils 80 and 83 and the mineral soils 96, 97, and 98. It is interesting to note that Appel and Wollenweber (1) mention this species as being more common in the soil itself than are the other *Fusaria*. Besides this, about a dozen of less constantly occurring Fusaria were isolated, nearly all from field and garden soils. Most common of these was a rarely fructifying, clamydospore-forming species, possibly *Fusarium falcatum*. Further *Fusarium culmorum* and several strains probably belonging to the variable *Fusarium solani*-group were met with.

The second group was represented by an unidentified *Phoma* species, which occurred in all field soils except three very acid ones, viz., 40, 44, and 45.

Besides these typical soil fungi a large number of species belonging to genera of less constant occurrence and difficult to identify were found. Of these can be mentioned: *Monilia* sp., common in moor and forest soils; *Cephalosporium* sp., common in soils of all types; *Botrytis cinerea*, occasionally found in field and forest soils; *Amblyosporium* sp., found in most forest soils; *Acrocylindrium granulosum*, common in soils of all types; *Spicaria* sp., fairly common in soils of all types; *Acrostalagmus cinnabarius*, found twice in field soils; *Mycogene nigra*, fairly common, especially in field soils; *Hormodendron cladosporioides*, fairly common in soils of very different character; *Sepedonium* sp., *Stachybotrys* sp., *Helminthosporium* sp., *Stemphylium* sp., and *Alternaria* sp., occasionally found in field and uncultivated mineral soils; *Ramularia* sp., fairly common in humus soils; and *Stysanus* sp., found once in field soil. Yeasts were met with occasionally, but they did not seem to be typical soil inhabitants [compare (46)]. Further several sterile mycelia and other not readily identifiable forms were isolated.

Thus upon the whole, the fungus flora observed here appears to be of the same general composition as that found by most other research workers (58). Its leading forms are Mucoraceae, Penicilliae, Trichodermae, and Fusaria, together with a number of species belonging to more sporadically occurring genera, mostly of hyphomycetes. Also the numbers of fungi are of the same general order of magnitude as those found in America by Waksman (54) and Starkey (45).

The numbers of fungi

The numbers of fungi vary, as table 1 shows, within wide limits from 24,300 (in 93) to 460,000 (in 54) per gram of soil. If any relationships exist between

these variations and definite soil characters, we may be justified in assuming that the numbers of fungi found by the plate method have a direct bearing on the abundance of fungi in the soil, although the numbers themselves refer only to the soil suspension and therefore depend largely on the method of preparing this, as shown by Brierley and his coworkers (4). Of such soil characters the following are here considered: physical texture of the soil, the humus content, the soil reaction, and the content of plant food.

The physical character exerts but little influence on the numbers of fungi, apart from the fact that all very heavy clay soils, viz., the five marsh soils 91 to 59 and the field soil 45 show remarkably low numbers of fungi—24,300 to 58,600 to the gram. Light sand soils, on the other hand, often have high numbers, such as soils, 1 to 6 and 30 to 32, in which the fungus flora consists mainly of Penicillia, which were very scarce in the marsh soils. It is quite natural, indeed, that the sand soils with their loose, open structure and good aeration would prove a better medium for the growth and fructification of these comparatively large, aerobic organisms than the heavy, sticky clays, of which the marsh soils further are more exposed to water-logging than any other group of soils.

The humus content does not show any definite relations to the fungus counts. Soils mainly consisting of organic matter, such as the moor soils and forest soils of mold or peat type, have not, upon the whole, higher numbers of fungi than the mineral soils. Several typical organic soils, such as the raw Sphagnum peats 74 and 75 and the forest soils 61 to 63, are even decidedly poor in fungi. Others, such as the forest soil 72 and the mud soil 84, have very high numbers.

The soil reaction is universally known to exert a profound influence on the microflora of the soil, and this is also the case here. It has, until the work of Ramann et al. (41), been nearly a dogma in soil microbiology that "fungi prevail in acid, bacteria in neutral and alkaline soils." Fischer (14) and Traaen (47) called attention to an occasional abundant occurrence of fungi in neutral and alkaline soils, and recently Waksman (58) has emphasized their activity in such soils.

The data in table 1, when all groups of soils are considered, show not the slightest correlation between the numbers of fungi and the corresponding pH values. Note, for example, the 10 most acid soils, nos. 65, 74, 67, 40, 44, 87, 73, 75, 45, and 68; and the 10 most alkaline soils, nos. 46, 3, 100, 98, 99, 34, 55, 56, 29, and 83.

The table further shows that the numbers of bacteria and actinomyces increase with increasing pH values, as would be expected *a priori*. There is, however, no very strict correlation between the reaction and the bacterial numbers, evidently because these are affected by many other factors besides the reaction. A quantitative expression of this correlation can be obtained by computing the correlation coefficient between the hydrogen-ion concentration and the numbers of bacteria plus actinomyces according to the formula of Branais

$$r = \frac{\Sigma\, dx \cdot dy}{n \cdot \sigma\, x \cdot \sigma\, y} \pm m = \frac{1 - r^2}{\sqrt{n}},$$

where r is the correlation coefficient, x and y the two covariates, *in casu* the hydrogen-ion concentration and the numbers of bacteria + actinomyces, dx and dy the individual digressions from the arithmetical mean, Σ the sign of summation, n the number of observations, σx and σy the standard deviations

TABLE 2

Comparison between pH values and ratios of fungi to bacteria + actinomyces in soils from table 1

NUMBER	pH	F.B+A*	NUMBER	pH	F:B+A	NUMBER	pH	F:B+A
65	3.34	0.63	81	5.64	0.037	7	7.03	0 0026
74	3.62	0.37	74	5.65	0.023	41	7.08	0 0067
67	3.65	0.85	35	5.92	0.018	50	7.15	0.0045
44	3.76	0.45	63	6.06	0.0082	13	7.15	0.0057
87	3.85	0.87	42	6.09	0.0092	10	7.16	0.0069
73	3.86	0.55	23	6.12	0.0026	43	7.20	0.016
45	3.97	0.36	24	6.14	0.0093	4	7.22	0.0069
88	4.10	0.46	20	6.22	0.0070	48	7.23	0.013
64	4.12	0.25	61	6.32	0.0078	14	7.26	0.0053
84	4.17	0.25	25	6.35	0.0099	12	7.32	0.0043
78	4.24	0.13	26	6.48	0.012	28	7.36	0.010
82	4.26	0.14	5	6.59	0.018	54	7.38	0.0045
66	4.32	0.13	15	6.70	0.0034	32	7.40	0.012
90	4.39	0.10	11	6.78	0.0098	21	7.50	0.010
75	4.45	0.099	17	6.79	0.0052	22	7.52	0.0043
77	4.58	0.21	91	6.82	0.0037	97	7.55	0.0046
38	4.96	0.056	23	6.85	0.0055	83	7.61	0.0031
59	4.97	0.052	16	6.86	0.0048	29	7.66	0.010
30	5.09	0.059	85	6.90	0.0029	56	7.75	0.0075
60	5.12	0.094	9	6.91	0.0039	55	7.78	0.0067
95	5.12	0.0077	6	6.91	0.0077	99	7.95	0.0035
76	5.14	0.015	27	6.92	0.0093	100	8.08	0.0026
80	5.20	0.030	31	6.93	0.016	46	8.35	0.0068
70	5.54	0.027	8	6.98	0.0045			
49	5.56	0.020	47	7.02	0.045			

* F. fungi; B = bacteria; A = actinomyces.

$\left(\text{calculated according to the usual formula } \sigma x = \frac{\sqrt{\Sigma dx^2}}{n}\right)$ of x and y, respectively, and m the standard error of the final result. It must be noted that these calculations have been made, not on the basis of the pH values, but on the corresponding molar hydrogen concentrations, since we must assume that it is the *absolute* concentration of hydrogen ions and not the logarithmic function thereof—namely, the pH value—which is of importance to the microörganisms living in the soil.

This calculation gives us:

$$r = -0.32 \pm 0.12$$

a negative correlation, which is significant, but not very marked. Although thus the actual numbers of fungi show no, and those of bacteria plus actinomyces only a comparatively small, correlation with the hydrogen concentration, the correlation of this with the *ratio of fungi to bacteria plus actinomyces* is very striking. Table 2 shows that this ratio is about 0.4 to 0.9 in the few soils of pH less than 4. When pH increases from 4 to 6 the ratio drops to 0.01 to 0.02, although with considerable variations. Above pH 6 there is no longer any correlation between pH and F: B + A, which here varies between 0.018 and 0.0026, except in the case of soil 47.

A better picture is obtained if we as before calculate the correlation coefficient between the hydrogen-ion concentration and the ratio F:B + A. This gives us:

In the group from pH 6.06 to pH 8.35: $r = -0.033 \pm 0.22$
In the group from pH 5.56 to pH 8.35: $r = +0.56 \pm 0.097$
In the group from pH 5.09 to pH 8.35: $r = +0.67 \pm 0.074$
In the group from pH 3.34 to pH 8.35: $r = +0.82 \pm 0.040$

There is thus above pH 6 no correlation whatever between hydrogen-ion concentration and F:B + A, but at pH 5.5 the correlation becomes positive and significant, and still more so from pH 5 to pH 3.3. Finally, if we consider the whole material we obtain a correlation so perfect that it is only rarely found in biological phenomena. We may conclude from this, that whereas the actual numbers of fungi and bacteria plus actinomyces depend on many factors, the ratio between these two groups is governed mostly by the hydrogen-ion concentration, except in soils of pH above 6.0, where the actual amounts of hydrogen ions seem to be too small to exert a distinct influence.

It is interesting to compare these result with those obtained by Waksman (54), who determined the numbers of bacteria, actinomyces, and fungi in variously treated plots of widely different pH values, from a permanent fertilizing experiment. The figures of his table 13 (54) show a very definite inverse correlation between pH and ratio of fungi to bacteria plus actinomyces, and a calculation of the correlation between hydrogen-ion concentration and F:B + A gives:

$$r = +0.83 \ (\pm 0.071)$$

i.e. practically the same value as the corresponding figure for the Danish soils.

Waksman's data further show an inverse relationship between pH values and the actual numbers of fungi to bacteria plus actinomyces, which was not observed in the present investigation. A calculation of the correlation between hydrogen-ion concentration and numbers of fungi in Waksman's table gives:

$$r = +0.65 \ (\pm 0.13)$$

The bacteria plus actinomyces, as in our results, increase generally with increasing pH values, and a calculation of the correlation between these numbers and the hydrogen-ion concentration gives:

$$r = -0.60 \ (\pm 0.17)$$

These figures are both significant, although the standard error is without significance in cases with so few observations (16).[4]

We see thus that the correlations of actual numbers to hydrogen-ion concentrations are good, but not so perfect as that of the ratio F:B + A, which was also the case with our results. That the actual numbers in Waksman's results were better correlated with the hydrogen-ion concentration than was the case with the Danish soils, is probably because Waksman's figures have been obtained from differently treated plots of the same soil, whereas the present data represent many different soils of widely varying character, where a much greater number of uncontrolled factors have been influencing the numbers of microörganisms. If differently treated plots of the same soil are compared, we do sometimes find a decrease in numbers of fungi with an increase of pH. Table 3 shows the data for soils from liming experiments, from which countings have been carried out.

TABLE 3

Influence of lime on numbers of fungi, bacteria, and actinomyces in field experiments

NUMBER OF SOIL	PROVENANCE	LIME PER HA.	pH	FUNGI PER GRAM OF SOIL	BACTERIA + ACTINOMYCES PER GRAM
		tons		*thousands*	*millions*
1	Askov Experiment	0	4.83	123.2	.....
2	Station	0	5.15	135.4	.....
3		32	8.10	127.0	.....
24	Borris Experiment	0	6.14	96.4	10.3
25	Station	2	6.35	104.8	10.6
26		4	6.48	113.4	9.9
27		8	6.92	104.4	11.2
28		16	7.36	112.0	11.00
29		32	7.66	109.6	10.5
30	Tylstrup Experiment	0	5.09	260.8	4.45
31	Station	8	6.93	207.4	12.6
32		32	7.40	198.6	17.0

In the Askov experiment, and also in the Borris experiment, where the pH values are all above 6.0, there is no effect of liming on fungi. In the Tylstrup experiment there is a small but noticeable reduction of the fungi, and a very great stimulation of the bacteria and actinomyces due to liming.

[4] According to Fisher (16) more than 100 trials would be necessary to obtain this correlation by random sampling from an uncorrelated population.

To test this point further, a series of liming experiments was carried out in the laboratory. Samples of soils of more or less acid reaction were divided into two parts, to one of which enough $CaCO_3$ was added to make its reaction

TABLE 4

Influence of liming on numbers of fungi in acid soils in the laboratory

SOIL AND TREATMENT	PERIOD OF INCUBATION	pH	FUNGI PER GRAM	BACTERIA + ACTINOMYCES PER GRAMS	ACTINOMYCES	RATIO F:B+A
	days		*thousands*	*millions*	*per cent*	
Heath soil (dry peat) no. 87, no addition	Start	3.85	183.0	0.21	None on casein agar	0.87
	15	4.26	494.0	0.72		0.68
	45	3.67	610.0	0.84		0.73
	75	3.90	910.0	1.47		0.62
Same soil + 4 per cent $CaCO_3$	Start	....	183.0	0.21	....	0.87
	15	7.52	420 0	83.1	22.0	0.0051
	45	7.47	393.0	398.0	21.1	0.0010
	75	7.59	595.0	415.0	29.6	0.0014
Low moor soil no. 82, no addition	Start	4.26	206.0	1.42	4.6	0.14
	15	4.10	202.0	1.08	3.1	0.19
	45	4.18	191.5	0.54	7.1	0.36
	75	4.13	223.0	1.16	....	0.19
Same soil + 4 per cent $CaCO_3$	Start	....	206.0	1.42	4.6	0.14
	15	8.05	262.8	60.8	2.6	0.0043
	45	7.50	201.5	96.5	2.5	0.0021
	75	7.76	298.0	161.0	10.9	0.0019
Sand soil no. 30, no addition	Start	5.09	260.8	4.45	40.8	0.059
	15	4.71	341.0	5.14	61.0	0.066
	90	4.42	547.0	4.61	57.6	0.12
Same soil + 1 per cent $CaCO_3$	Start	....	260.8	4.45	40.8	0.059
	15	7.57	365.0	22.9	35.0	0.016
	90	7.50	605.0	43.7	23.4	0.014
Light loam no. 35, no addition	Start	5.92	131.4	7.40	32.0	0.018
	15	6.04	148.0	10.6	24.0	0.014
	50	5.78	126.8	7.87	36.2	0.017
Same soil + 1 per cent $CaCO_3$	Start	5.92	131.4	7.40	32.0	0.018
	15	7.72	72.0	19.2	15.5	0.0037
	90	7.62	120.0	17.1	20.4	0.0055

distinctly alkaline. Enough distilled water was added to saturate it to 70 per cent of its water-holding capacity, and the samples were kept at 25°C. in glass dishes, covered with Petri dish tops, for up to 120 days, during which the moisture content was kept as constant as possible by the addition of dis-

tilled water, and at various intervals of time counts of fungi, bacteria, and actinomyces were made.

The results (table 4) agree well with the previous ones. In the heath soils there is a relative depression of the numbers of fungi: there active multiplication, probably because of the alteration of the structure and the better aeration of the soil, reaches a considerably smaller extent in the limed than in the unlimed soil. In the moor and the sand soil the fungi seem to be stimulated by the liming. In the loam soil, finally, there is first a decrease of fungi in limed soil, later followed by a corresponding increase. The bacteia and actinomyces are strongly affected. Although their numbers do not vary much in the untreated soils, they undergo in the limed soils a rapid multiplication, which in the heath soil—a mixture of coarse sand and peat, rich in undecomposed plant residues—is enormous, from 0.2 to 415 millions to a gram of soil. In the two less acid mineral soils their multiplication, although vigorous, is far less pronounced, probably because the amount of microbial food, which the liming renders available to the bacteria, is smaller in these soils, where a fairly abundant bacterial flora has been active before the liming.

The last column of the table shows a ratio of F:B+A of quite the same order of magnitude as in soils of corresponding reaction taken directly from the field. The agreement with the natural conditions is indeed so good that when the coefficient of correlation between hydrogen-ion concentration and F:B+A in the 24 counts[5] presented in table 4 is calulated, we obtain:

$$r = 0\ 81 \pm (0\ 07)$$

This shows that the abundance of fungi, in contact to that of bacteria and actinomyces, as determined by the plate method, is not much influenced by liming of the soil. One gets the impression that the fungi as a whole do not prefer acid reaction, although it is a commonplace that they are much more independent of the reaction of the medium than are the bacteria and actinomyces. Experiments were made to determine the ability of some common soil fungi, actinomyces, and bacteria to grow at different hydrogen-ion concentrations. This is a field in which we possess definite knowledge for only a comparatively small number of soil organisms, since most research in this direction has been devoted to technically and pathologically important microorganisms rather than to ordinary soil saprophytes.

A number of fungi were grown in the following medium: dextrose, 10 gm.; asparagine, 1 gm.; KH_2PO_4, 2 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.5 gm.; $FeCl_3$, 0.1 gm.; H_2O, 1,000 cc. Portions of this solution were titrated by means of hydrochloric acid and sodium hydroxide to pH values ranging from 1.2 to 8.8. The solutions were then distributed in 10-cc. portions to Jena glass test tubes and sterilized, whereupon the reaction was measured again. Duplicate cultures were incubated at 25°C. until no further change in the amount of growth was observed. Table 5 gives the results.

[5] The counts at the start are common for limed and unlimed soil.

The fungi show a considerable difference in their tolerance toward acidity. The extremes are represented by *Penicillium* sp. I and *Trichoderma* sp. V, which still grow at pH 1.4, and by *Coccospora* sp. (mentioned later) which fails to grow between pH 3.7 and 4.3. A distinct optimum at acid reaction is seen only in *Mucor ramannianus*, *Absidia cylindrospora*, and *Trichoderma* sp. V. One of the Fusaria shows some indication of a double optimum, as observed by Hopkins (20) and Lundegårdh (29). It is further seen that all fungi except *Mucor ramannianus* are able to start growth at pH 8.8, a degree of alkalinity exceptional in Danish soils; pH 3.1 all fungi except *Coccospora* and *Phoma* sp. are able to grow (it is noteworthy that the few field soils in which the markedly acid-sensitive *Phoma* was not found, were the strongly acid soils

TABLE 5

Growth of various soil fungi at different reactions

ORGANISM	GROWTH AT pH:													
	1.16	1.42	2.01	2.53	3.83	3.10	3.71	4.27	5.52	6.02	6.57	7.19	7.86	8.8
Mucor hiemalis	0	0	0	2	2	3	3	4	4	4	4	4	4	4
Mucor ramannianus	0	0	0	1	...	2	...	3	...	3	3	2	1	0
Zygorhynchus sp.	0	0	0	0	...	3	3	3	...	3	3	2	2–3	1–2
Sterile Phycomyc.	0	0	0	0–1	...	1–2	...	3	...	3	3	3	3	2
Absidia cylindrospora	0	0	1	5	5	5	5	5	5	4	3	3	3	3
Trichoderma I	0	0	0	0–1	...	...	2	2	...	2	3	3	2	2
Trichoderma V	0	1	3	3	3–4	4	5	5	4	3	3	3	3	3
Penicillium sp. I	0	1	2–3	2–3	...	...	...	3	3	3	3	3	3	3
Citromyces sp.	0	0	2	3	3	4	5	5	5	5	5	5	5	5
Aspergillus fumigatus	0	0	1	1	4	4	5	5	5	5	5	5	5	4
Coccospora agricola	0	0	0	0	0	0	0	2	4	4	4	4	4	4
Mycogone nigra	0	0	0	0	0	1–2	...	3	...	3	3	3	3	2
Fusarium orthoceras	0	0	1	1–2	1–2	2	...	3	...	4	4	3	3–4	3–4
Fusarium (falcatum?)	0	0	0	0–1	1–2	2–3	2–3	5	3	4	5	5	5	5
Phoma sp.	0	0	0	0	0	0	1	2	3	3	3	3	3	3

The growth here and in the following tables is indicated by the characters: 0 = no growth, 1 = trace, 2 = scant, 3 = fair, 4 = good, 5 = excellent growth.

40, 44, 45, of pH 3.7 to 3.9), and in this whole interval, within the limits of which the pH values of nearly all Danish soils lie, most fungi grow equally well. This is in good agreement with the results of Johnson (5), who found *Mucor glomerula* able to develop in the interval from pH 3.2 to 3.4 to pH 8.7 to 9.2, and 6 species of *Penicillium*, *Aspergillus*, and *Fusarium* would grow within the limits of pH 1.6 to 2.2 to pH 9 to 11.2. Within these limits there was a very broad optimal zone which had its greater extent on the acid side of the point of neutrality. Similar figures are given by Hopkins (20), McInnes (31) and Lundegårdh (29) for *Fusaria* (maximal acidity pH 2.4 to 3), by Currie (10) for *Aspergillus niger* (pH 1.4 to 1.6), and by Meacham (34) for wood-destroying basidiomycetes (pH 1.7 to 2). Melin (35) found the

saprophytic soil fungi *Rhizoctonia silvestris* and *Mycelium radisis atrovirens* able to grow equally well at pH 3.4 and 7. Some parasitic and symbiotic fungi behave differently; Melin (35) thus found mycorrhiza-forming species of *Boletus* growing best in pure cultures at pH 5, with a distinct decline on both sides. Weis and Nielsen (67) found that *Polyporus radiciperda* has a distinct optimum at pH 4 to 4.5 and produces only a very slight growth at pH 5.9 to 6.1. We thus get the general impression that the great majority of ordinary saprophytic soil fungi are quite tolerant toward the reaction of the medium,

TABLE 6

Growth of soil actinomyces at different reactions

ORGANISM	GROWTH AT pH:					
	7.34	5.98	5.40	5.16	4.88	4.41
Actinomyces griseus	4–5	5	5	4	0–3	0
Actinomyces griseoflavus	3–4	4–5	5	5	1–2	0
Actinomyces cellulosae	4–5	4–5	4–5	4–5	1	0
Actinomyces fulvissimus	2	2–3	3–4	5	4	3–4
Actinomyces violaceus-ruber	3–4	4–5	3–4	3–4	3–4	0–3
Actinomyces diastatochromogenus	...	5	2	2	0	0
Actinomyces phaeochromogenus	2–3	2	2	2	1	0

TABLE 7

Growth of 10 soil bacteria in broth of varying reaction

ORGANISM	GROWTH AT pH:									
	2.58	3.58	3.88	4.08	4.36	4.58	4.78	5.26	6.06	7.27
Bac. mycoides	0	0	0	0	0	0	0	2	4	4
Bac. megatherium	0	0	0	0	0	0	1–2	1–2	2	3
Bacillus III	0	0	0	0	0	0–1	1	1	1	1
Bacillus IV	0	0	0	0	0	0	0	1	3	2–3
Bact. coeruleum (?)	0	0	0	0	0	0	0	2	4	4
Non-spore-forming rod I	0	0	0	0	0	0	0	0	2	2
Non-spore-forming rod II	0	0	0	0	3	4	4	4	4	4
Non-spore-forming rod III	0	0	0	0	3	4	4	4	4	3
Non-spore-forming rod IV	..	0	0	0	0	0	0	1	3	4
Mycobacterium sp.	0	0	0	0	0	0	0–1	4	3–4	2–3

and their preference for acid reaction is not always clearly pronounced. It is therefore not surprising that no direct relationship can be traced between their abundance and the soil reaction.

Quite reverse features are presented by the *actinomyces* and *bacteria*. Seven strains of common soil actinomyces were grown in the following medium: soil extract (1 kgm. of soil + 1 liter of tap water heated for 30 minutes, in the autoclave) 1,000 cc.; soluble starch, 10 gm.; asparagin, 2 gm.; K_2HPO_4, 2 gm.; reaction varied by means of HCl to pH values between 4.4 and 7.4. The solution was used in 5-cc. portions in test tubes, and duplicate cultures were

incubated for 14 days at 25°C. The results (table 6) confirm those of Waksman and Joffe (59) and Waksman (55), and of the author in another paper (24): most of the typical soil actinomyces are checked in their growth at pH 4.8 to 5.1, although a few grow at pH 4.4 and still lower.

A number of soil bacteria were isolated at random from various soils in table 1, and tested for their resistance to acidity in the following medium: meat extract, 5 gm.; peptone, 10 gm.; glycerin, 10 gm.; sodium citrate, 4 gm.; K_2HPO_4, 2 gm.; H_2O, 1,000 cc. After being clarified, the medium was titrated to pH values ranging from 2.6 to 7.3. The results are presented in table 7 and show plainly that the growth of the majority of the bacteria is checked in the pH interval 4.8 to 5.7. Only two bacteria from acid soil (nos. 60 and 87) continue to grow at pH 4.36. These results are much the same as found by several other investigators for a number of soil bacteria, as shown in the following:

Organism	Maximal acidity *pH*	Reference
Azotobacter chroococcum	5.6–6.0	Various authors cited after Waksman (58)
Rhizobium radicola	4.3–5.0	
Rhizobium beijerinck	3.2–3.4	
Clostridium amylobacter	5.7	
Clostridium putrificum	5.8	
Bacillus subtilis	4.2	
Bacterium coli	4.4	
Bacterium stutzeri	6.1	
Pseudomonas pyocyanea	5.6	
Spirochaete cytophaga	5.2–5.7	Dubos (13)
Cellulose decomposing vibrios	6.0–6.5	
Unidentified bacteria from peat: 7 strains	5.0	Itano (21)
Unidentified bacteria from peat: 1 strain	6.0	
Unidentified bacteria from peat: 1 strain	4.0	
Unidentified bacteria from peat: 1 strain	3.0	

We must bear in mind that the pH limits found in artificial culture do not necessarily apply strictly to the natural conditions in the soil: firstly, because an organism may show a larger range of tolerance to acidity in its natural habitat, and secondly, because local differences in reaction, perhaps also symbiotic phenomena, may enable an organism to lead an active life in a soil, where the reaction is, *per se*, prohibitive to the development of the organism in question. But still the results show an essential difference between the two groups of soil microörganisms—fungi vs. bacteria and actinomyces which helps us to account for the interesting correlation between reaction and ratio of fungi to bacteria. Besides the well-known fact that bacteria (and actinomyces) are generally benefited by a neutral to alkaline reaction, we observe that the pH interval 5 to 6 appears critical, or at least unfavorable, to the great majority of soil bacteria and actinomyces. And this is just the region of the scale of reaction where the correlation betwen hydrogen-ion concentration and ratio of fungi to bacteria plus actinomyces becomes significant, as has been shown.

The food supply would of course *a priori* be expected to exert a considerable

influence upon the abundance of fungi. A direct relation between the numbers of fungi in the soils and their fertility in agricultural respect cannot, however, be traced. The fertile loamy soils from Tystofte (nos. 15–17), Aarslev (nos. 22–23), and Lolland (nos. 20–21) have, for instance, lower numbers than the poor sand soils from Askov (nos. 1–11) and Tylstrup (nos. 30–32), and the raw heath soils 37–38. It seems, upon the whole, impossible to compare soils of different character and origin, but if we compare differently treated plots of the same soil, we see a certain influence of fertilization. By collecting the data from soils from fertilization experiments in table 1 we obtain the results given in table 8.[6]

TABLE 8

Influence of fertilization on numbers of microorganisms in field experiments

SOIL NUMBER	PROVENANCE	TREATMENT	FUNGI, PER GRAM OF SOIL	BACTERIA + ACTINOMYCES PER GRAM
			thousands	*millions*
4	Askov Experiment Station, Sand Field	Unmanured	92.4	13.4
5		Mineral fertilizer	218.0	12.0
6		Farmyard manure	166 6	21.6
7		Unmanured	41 4	15.7
8		Mineral fertilizer	79 0	17.6
9		Farmyard manure	87.2	22.5
12	Askov Experiment Station, Loam Field	Unmanured	68 8	16.1
13		Mineral fertilizer	100 4	17 6
14		Farmyard manure	109.4	20.5
18	Lyngby Experiment Station	Unmanured	63.0	....
19		Farmyard manure	80 8	....
33		Unmanured	86.4	....
34		Farmyard manure	154 6	....
22	Aarslev Experiment Station	Unmanured	78.0	16 2
23		Farmyard manure	132.0	24.0

There is here a significant increase in numbers of fungi (besides in bacteria and actinomyces) with the addition of fertilizer and especially farmyard manure, as previously found by Fischer (15) and Waksman (54). Besides this, there is another way which enables us to trace the connection between the abundance of fungi and the food supply of the soils, viz., the calculation of the correlation between numbers of fungi and bacteria plus actinomyces. We may assume that the soils with a higher supply of available microbial food will, other things equal, show a higher number of bacteria than soils with a lower supply; and the numbers of bacteria determined by the plate·method is a

[6] Some of these data have been previously published in a paper by Christensen (7), together with other counts of bacteria in soils from fertilizing experiments.

reliable index of the density of the bacterial population (16), although it does not give the absolute figures.

If we select the 45 soils with pH values above 6 (where the reaction does not markedly influence the numbers of bacteria and actinomyces) from table 1 and calculate the correlation coefficient between numbers of fungi and bacteria plus actinomyces, omitting the somewhat unusual garden soil 54 with its extraordinarily high numbers of both fungi and bacteria, we obtain:

$$r = +0.41 \pm 0.13$$

This value, which is not very high but which is significant, shows fairly clearly that the factors which favor the existence of high numbers of bacteria and actinomyces in the soil, have a general tendency also to favor the high numbers of fungi. Had soil 54 been included in the calculation, the correlation would have become still better.

It is interesting to compare this result with some data given in a recent paper by Starkey (45), who determined the abundance of fungi, bacteria, and actinomyces at different time in planted and unplanted field and greenhouse soils. The field soil was a neutral loam, and counts were made at five periods in fallow soil and under eight different crops. These 45 counts give us the following correlation between numbers of fungi and bacteria plus actinomyces:

$$r = +0.43 \pm 0.12$$

practically the same as was found here. The greenhouse soil was a heavy clay of pH 5.5. Counts were made at six periods in fallow soil and under five different crops. These 36 counts give:

$$r = +0\ 32 \pm 0.15$$

a correlation which is just noticeable, since it would, according to Fisher (16), be obtained by chance once in about 20 trials.

The Importance of Fungi in Various Decomposition Processes

The data obtained by the simple plate method have only a limited value and do not allow a comparison between the actual amounts of fungus mycelium in the soils; they should therefore, if possible, be combined with other experiments. The important thing is not merely what numbers of fungi can we obtain from the various soils, but also, to what extent are the fungi capable of multiplying, when some process of decomposition is going on in the soil? To obtain an answer of this question it would be of interest to see how different treatments affect the fungus and bacterial flora in different soils, such as has previously been done by Waksman and co-workers (60, 61, 64) in studies of partial sterilization of soils and decomposition of organic matter. It is this principle, which Winogradsky (69) has introduced methodically as *dynamic microbiology.*

The experiments were carried out in the same manner as the liming experiments mentioned above. The following substances were tested: Nitrogen-free compounds, dextrose and cellulose; nitrogenous materials, casein and alfalfa seed meal.

DECOMPOSITION OF NITROGEN-FREE COMPOUNDS

Dextrose decomposition experiments were carried out with the following soils:

1. Low moor soil from Tylstrup, pH 4.8
2. Forest sand soil from Lyngby, pH 5.1
3. Loamy field soil from Lyngby pH 5.9
4. Sand soil from Askov, pH 6.8
5. Sand soil from Borris, pH 7.7

Dextrose was added in a concentration of 0.5 per cent on a basis of air-dry soil, and enough distilled water to saturate 75 per cent of the water-holding capacity was added. Counts were carried out after 6 and 12 days at 25°C.

TABLE 9

The multiplication of fungi and bacteria in soils of different reactions with addition of dextrose

SOIL	FUNGI PER GRAM			BACTERIA + ACTINOMYCES PER GRAM		
	Start	6 days	12 days	Start	6 days	12 days
	thousands	*thousands*	*thousands*	*millions*	*millions*	*millions*
Low moor soil, pH 4.78	124.0	4,560 0	30,280.0	0 55	5.86	7.60
Sandy forest soil, pH 5.07	60 0	2,850.0	8,340.0	1 50	34.4	16.8
Light loam, pH 5.92	131.4	157 0	323.0	7.40	.....	121.0
Field soil, sand, pH 6.78	145 6	166.0	......	14.9	206 5	.....
Field soil, sand, pH 7.66	109.6	290.0	225.0	10.5	71.0	92.0

The results in table 9 are quite striking. The neutral to alkaline soils 4 and 5 show a large increase in the numbers of bacteria (but not of actinomyces), and the fungi are but little affected. On the other hand, in the two distinctly acid soils 1 and 2, the fungi increase greatly, especially after 12 days (perhaps spore formation is taking place at this period) but the bacteria and actinomyces multiply only to a small extent. The fungi which multiplied most markedly were *Mucor spinosus* (?) and Penicillia in the moor soil, *Mucor Ramannianus* and Penicillia in the forest soil. These observations agree partly with those of Waksman and Starkey (60), who also found that a dextrose addition stimulates the bacteria greatly in neutral but not in strongly acid soil. Contrary to the case here, they found no marked influence of dextrose on the abundance of fungi in acid soil, probably because a period of incubation of only two days was used. Table 9 further shows the noteworthy fact that this marked change in the multiplication of the fungi occurs in the pH interval 5.1 to 5.9—the region where the influence of reaction on the ratio of fungi to bacteria and actinomyces becomes noticeable, and where most soil bacteria

and actinomyces begin to be unfavorably influenced by the reaction, as has been shown.

Finally an experiment was run to determine whether the sugar was decomposed more rapidly by fungi in a soil of acid reaction or by bacteria in a soil of alkaline reaction. An acid, sandy forest soil, fairly rich in organic matter, was divided into two portions, to which were added distilled water as before, and to one, 1 per cent $CaCO_3$. They were then kept at 25°C. for eight days, after which counts of microörganisms were made. One per cent of dextrose was then added, and determinations of the evolved CO_2 were carried out for six days ar 25°C. by means of the method developed by Petersen (40). At the end of the experiment, counts of microörganisms were again made. The results are found in table 10.

The dominating fungi were Penicillia and *Zygorhynchus* sp. in the unlimed, and *Monilia* sp. in the limed, soil. Since all dextrose had disappeared after

TABLE 10

Decomposition of dextrose in acid soil with and without lime

		SOIL $CaCO_3$	SOIL + $CaCO_3$
Before experiment	Fungi per gram . . . *thousands*	109.2	108.0
	Bacteria + actinomyces per gram . . . *millions*	2.2	34.5
	Actinomyces . . . *per cent*	61.1	2.4
	pH	4.48	7.40
Total production of CO_2, mgm. per 100 gm. of fresh soil		543.1	735.0
After experiment	Fungi per gram . . . *thousands*	21,000.0	980.0
	Bacteria + actinomyces per gram . . . *millions*	56.2	1,268.0
	Actinomyces . . . *per cent*	45.6	0.4
	pH	5.01	7.27
	Dextrose in soil	None	None

six days and the pH values do not indicate any formation of organic acids, it seems that the sugar has been equally rapidly decomposed in both soils, and that the smaller production of carbon dioxide in the acid soil is due to the extensive synthesis of fungus mycelium which has taken place in this soil.

Decomposition of cellulose

There is much evidence that the decomposition of cellulose and related compounds is one of the chief functions of the fungus flora of the soil. Van Iterson (22) and Koning (26) found a large number of fungi able to grow on filter paper, and nearly all later investigators have pointed out the striking ability of these organisms to utilize cellulose [Traaen (47), Daszewska (11), McBeth and Scales (30), Otto (39), Waksman and co-workers (48, 61, 63, 64), Rege (42) and others].

In the present experiments four soils of different character were used.

TABLE 11

Multiplication of fungi in soils with addition of cellulose

SOIL AND TREATMENT	FUNGI PER GRAM OF SOIL						
	0	15 days	30 days	45 days	60 days	90 days	120 days
	thousands	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*
1. Sand soil, no. 11, with 1.0 per cent cellulose, pH 6.78	145.6	142.0	....	....	....	148.5	106.0
2. Same +0.2 per cent $(NH_4)_2SO_4$ nitrified, pH 4.82	101.5	4,085.0	14,400.0	34,600.0	....	29,500.0	23,700.0
3. = 2, with addition of 2 per cent $CaCO_3$, pH 7.3	101.5	334.0	530.0	293.0	....	410.0	390.0
4. Loam soil, no. 16, with 1.0 per cent cellulose, pH 6.86	80.0	149.2	230.0	245.6	247.0	260.0	173.6
5. Same +0.2 per cent $(NH_4)_2SO_4$ nitrified, pH 4.56	61.4	3,850.0	6,420.0	7,110.0	....	7,000.0	8,400.0
6. = 5, with addition of 2 per cent $CaCO_3$, pH 7.3	61.4	450.0	427.0	145.0	158.8	375.0	287.0
7. Sand soil, no. 27, with 1.0 per cent cellulose, pH 6.92	104.4	261.0	284 0	245.0	205.0	143.5	....
8. Same +0.2 per cent $(NH_4)_2SO_4$ nitrified, pH 4.60	121.4	5,665.0	30,500 0	36,000.0	45,000.0	54,000.0	....
9. = 8, with addition of 2 per cent $CaCO_3$, pH 7.4	121.4	460.0	2,666.0	3,130 0	3,200.0	2,310.0	....
10. Loam soil, no. 35, with 1.0 per cent cellulose, pH 5.92	131.4	135.0	460.0	567.0	470.0	353.0	....
11. Same +0.2 per cent $(NH_4)_2SO_4$, not nitrified, pH 5.92*	131.4	1,600.0	14,500.0	37,700.0	21,2000	26,900.0	....
12. = 11, with addition of 2 per cent $CaCO_3$, pH 7.2	131.4	1,200.0	720 0	795.0	518.0	....	....

* Changed to pH 4.8–4.5 during course of experiment.

They received 1 per cent cellulose as ground filter paper, and distilled water to 70 per cent of the water-holding capacity, in some cases also a source of N. By choosing $(NH_4)_2SO_4$ for this, allowing it to nitrify until the soil had reached a pH value of 4.8–5.0, dividing the soil in two portions, and adding $CaCO_3$ to one of them, samples of the same soil with different reactions were obtained. The multiplication of the fungi was then determined during a period of 90–120 days at 25°C. Table 11 gives the result.

We see that the addition of cellulose alone to the soil causes a certain multiplication of the fungi, but much more striking results are obtained when combined N is also added [compare Waksman and co-workers (61, 64)]. In the acid soils, nos. 2, 5, 8, and 11, the fungi multiply enormously and are here represented almost exclusively by Penicillia and Trichodermae. In the neutral and alkaline soils, nos. 3, 6, 9, and 12, the fungi develop vigorously too, although the counts do not run nearly so high as in the acid soils [compare Waksman and Heukelekian (61), and Dubos (12)]. But the most interesting thing is *that quite a different fungus flora arises at neutral to alkaline reaction.* It consists almost entirely of *Mycogone nigra, Acrocylindrium granulosum,* and a fungus closely resembling the *Coccospora agricola* described by Goddard (17). All these forms were present in only small numbers or (esp. *Coccospora*) were not noticeable in the original soil flora. It would now be of interest to see how the different fungi utilize cellulose in pure culture. A series of qualitative experiments were carried out by growing soil fungi on cellulose as a strip of pure Swedish filter paper in test tubes with the following solution: NH_4NO_3, 2 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 0.5 gm.; H_2O, 1,000 cc. Cultures were incubated for 30 days at 25°C. The results in table 12 show that the Mucoraceae cannot utilize cellulose. These results are in agreement with those found previously by Hagem (19) and by Waksman and Heukelekian (63), and with the fact that they were not richly represented in the flora which develops in the soil with addition of cellulose, as also noted by Waksman and Stevens (65). The Hyphomycetes are quite different: here the inability to utilize cellulose is a rather rarely occurring exception (*Monilia* sp., *Aspergillus repens*, *Sepedonium* sp., sterile mycelium II), and it is especially noteworthy that the two fungi which were the most prominent in the counts from neutral and alkaline soils with cellulose, viz., *Mycogone nigra* and *Coccospora agricola*, are among the very strongest cellulose decomposers. The *Mycogone nigra* found here corresponds well to the description of this organism by Jensen (23) and also shows similarity to the genus *Humicola,* which Traaen (47) and Waksman (57) mention as an active cellulose decomposer. Van Iterson (22) and Otto (39) also have examined species of *Mycogone,* which they both mention as the strongest cellulose decomposing organism studied by them. The prevalence of the *Penicillium-Trichoderma*-group at acid, and of the *Mycogone-Coccospora*-group at neutral and alkaline, reactions agrees with what we found in table 5—that the latter fungi are rather sensitive to acidity and grow well at neutral to alkaline reaction, whereas those of the former group are very resistant to acidity and

may show a definite optimum at acid reaction. It also agrees perfectly with the results of Dubos (13), who found *Penicillium* sp. and *Trichoderma koningi* able to grow on filter paper at pH values between 2.1 to 3.0 and 9.0 to 9.5, with an optimum at pH 3.5 to 5.0 for *Penicillium* and 3.06 to 5.5 for *Trichoderma*, whereas *Humicola* sp. (=*Mycogone nigra?*) would grow from pH 3.0 to 9.5 with an optimal zone from pH 3.5 to 8.5.

To determine whether this difference is also apparent in the activity of the two groups of fungi in decomposing cellulose at acid and alkaline reactions, a quantitative cellulose decomposition experiment was carried out. A light

TABLE 12

Growth of various soil fungi on filter paper cellulose

ORGANISM	GROWTH	ORGANISM	GROWTH
Mucor hiemalis	0–1	*Penicillium* XXII	4
M. circinelloides	0–1	*Amblyosporium* sp.	4
M. Ramannianus	0	*Botrytis cinerea*	5
M. spinosus	0	*Monosporium* sp.	4
Zygorhynchus sp.	0–1	*Sepedonium* sp.	0
Absidia cylindrospora	0–1	*Acrocylindrium granulosum*	3
Cunninghamella elegans	0–1	*Coccospora ?*	5
Monilia sp.	0	*Acrostalagmus cinnabarinus*	3
Cephalosporium sp.	2	*Citromyces glaber*	3
Trichoderma I	4	*Spicaria* sp.	4
Trichoderma II	3	*Mycogone nigra*	5
Trichoderma III	1	*Hormodendron cladosporioides*	3
Trichoderma IV	3	*Stachybotrys* sp.	4
Trichoderma V	5	*Stemphylium* sp.	4
Aspergillus repens	0	*Stysanus stemonites*	3
A. fumigatus	3	*Fusarium solani*	3
Penicillium I	4	*F. orthoceras*	2
Pencillium II	3	*F. culmorum*	3
Pencillium IV	3	*Fusarium* sp. X	5
Pencillium V	3	*Ramularia* sp.	3
Penicillium IX	3	Sterile mycelium I	3
Penicillium XV	3	Sterile mycelium II	0
Penicillium XVI	3	Sterile mycelium II	1
Penicillium XVII	4	Sterile mycelium IV	5

sandy field soil, poor in organic matter, from the Tylstrup Experiment Station was used. The soil received—on an air-dry basis—1 per cent cellulose as ground filter paper, and 25 per cent of the following solution: NH_4NO_3, 5 gm.; KH_2PO_4, 5 gm.; *N*-HCl, 8 cc.; H_2O, 1,000 cc. This gave the soil a pH value of 4.5 (originally 4.8). Portions of 120 gm. of moist soil were placed in round 300-cc. flasks stoppered with perforated rubber stoppers, in which the hole was filled with cotton wool. The flasks were then sterilized in the autoclave, and in half of them the soil was made alkaline after sterilization by the addition of sterile $CaCO_3$. They were then inoculated with pure cultures of *Penicillium*

sp. I, *Trichoderma* sp. V, *Mycogone nigra,* and *Coccospora agricola.* For comparison a corresponding experiment was run with unsterilized soil. After 30 days' incubation at 25°C. determinations of residual cellulose by the method of Charpentier (6) showed the results reproduced in table 13 (average of two parallels).

These results are in perfect agreement with the previous ones and with those of Dubos (13): *Penicillium* and *Trichoderma* show a stronger activity at acid reaction, though *Trichoderma* is but little influenced. *Mycogone* and especially *Coccospora* are very distinctly favored by an alkaline reaction. It is further seen that the natural soil flora decomposes the cellulose more rapidly

TABLE 13

Decomposition of cellulose by fungi in acid and alkaline soils

ORGANISM	PER CENT OF ADDED CELLULOSE DECOMPOSED IN	
	Acid soil (pH 4.5)	Alkaline soil (pH 7.6)
Uninoculated	0	0
Penicillium sp. I	62	32
Trichoderma sp. V	80	70
Mycogone nigra	40	78
Coccospora agricola	23	66
Unsterilized soil	85	91

TABLE 14

Fungi, bacteria, and actinomyces in acid and alkaline soil plus cellulose

	FUNGI PER GRAM	BACTERIA + ACTINOMYCES PER GRAM	ACTINOMYCES
	thousands	*millions*	*per cent*
Soil before experiment	41.0	0.97	10.9
Soil after experiment:			
Without $CaCO_3$	1,360.0	8.6	2.3
With $CaCO_3$	178.0	71.4	1.6

than any of the pure cultures, although *Trichoderma* in acid soil and *Mycogone* in alkaline soil are not much behind. It also appears, in agreement with the results of Charpentier (6), Barthel and Bengtsson (2), and Waksman and Heukelekian (61), that cellulose decomposition is approximately equally rapid with and without addition of lime to acid soil.

Counts of microörganisms were carried out in the unsterilized soils before and after the experiment, with the results shown in table 14.

In the acid soil the dominating fungi after the experiment were *Penicillium* sp. XVII (*purpurogenum?*) and *Mucor ramannianus;* in the alkaline soil, *Mycogone nigra* and *Mucor ramannianus.* The results thus agree with the

previous ones, except for the abundant development of *Mucor ramannianus*. It is further seen, in agreement with Waksman and Starkey (60), and Waksman and Skinner (64), that the bacteria and actinomyces are not much affected by the addition of cellulose; the strong multiplication of these organisms in the alkaline soil may simply be due to the action of the lime (compare table 4).

The fungi are thus active in the decomposition of cellulose both at acid and alkaline reactions. Under natural conditions they are probably less important in neutral and alkaline soils, since there they must share the energy material with cellulose decomposing bacteria, which are strongly active under these conditions (12), and the cellulose is not decomposed much more rapidly in this case than at an acid reaction, where the cellulose is at the disposal of the fungi alone. But one thing must be kept in mind: the differences in numbers of fungi in acid and alkaline soils in the experiments mentioned do not indicate corresponding differences in activity, since the abundantly sporulating Penicillia and Trichodermae must necessarily show figures of quite another order of magnitude than *Mycogone* and *Coccospora*, which produce only comparatively few chlamydospore-like conidia.

DECOMPOSITION OF NITROGENOUS MATERIALS

Müntz and Coudon (38), and Marchal (33) observed that soil fungi have a marked capacity of decomposing nitrogenous organic materials with production of ammonia, and Butkewitch (5) first called attention to their marked proteolytic activity. Since then a considerable amount of work (8, 27, and 48) has been devoted to the study of the "ammonifying power" of soil fungi. Waksman (51, 62) pointed out that the accumulation of ammonia is not an index of proteolytic activity and therefore not a fair basis for the comparison of microörganisms of different proteolytic powers. Organisms which produce little ammonia may produce much amino nitrogen, and ammonia accumulation is rather an index of the utilization of nitrogenous compounds as energy material and therefore entirely dependent on both the nature of the microorganisms and the carbon-nitrogen ratio of the decomposed material.

Since the correctness of these arguments was recognized, no "ammonification experiments" were carried out, but an attempt was made to determine the importance of fungi in protein decomposition by adding nitrogenous materials to soils of various character, isolating the fungi which were specially stimulated, and testing their proteolytic activity.

Decomposition of casein

The first set of experiments was carried out with casein as an example of a pure protein. Four soils were used; they received 1 per cent casein, and water to 75 per cent of water-holding capacity, in some cases also 2 per cent cellulose. Counts of microörganisms were made over a period of 45 days. Table 15 shows the results.

The fungi multiply enormously in the two strongly acid soils, and in no. 2

there is an equally rapid decrease after 30–45 days, so that at this period the fungi disappear almost entirely. Still higher and more consistent figures are obtained when cellulose is also added. The composition of the flora is interesting: it consists in no. 1 almost entirely of *Mucor hiemalis*, *Mucor ramannianus*, and sterile Phycomycetous mycelium (*Cunninghamella elegans?*); when cellulose too is added, *Mycogone nigra* appears in large numbers. In no. 2 the flora both with and without cellulose consists of *Mucor ramannianus* and

TABLE 15

Multiplication of fungi, bacteria, and actinomyces in soils during decomposition of casein

SOIL AND TREATMENT		START	AFTER 10 DAYS	AFTER 20 DAYS	AFTER 30 DAYS	AFTER 45 DAYS
1. Sandy forest soil, pH 4.95, + 1 per cent casein	Fungi	236.5	2,360.0	7,040 0	7,325 0	.....
	B + A	4.77	533.2	538.8	510.0	.....
	A. per cent	13.3	2.4	20 2	13.7	.....
1a. Same + 2 per cent cellulose	Fungi	236.5	14,640.0	12,300.0	10,850.0	.....
	B + A	4.77	2,850.0	4,310.0	322.0	.....
	A. per cent	13.3	1.8	2.4	14.4	.....
2. Heavy clay, no. 38, pH 4.96, + 1 per cent casein	Fungi	86.0	27,000.0	8,850.0	......	37.0
	B + A	1.86	81.5	86.1	765.0	76.7
	A. per cent	22.2	30.9	4.6	3.2	4.8
2a. Same + 2 per cent cellulose	Fungi	86.0	71,000.0	88,000.0	94,600.0	.....
	B + A	1.86	63.5	344.4	242.2	.....
	A. per cent	22.2	35.5	45.2	57.1	.....
3. Light loam, no. 35, pH 5.92, + 1 per cent casein	Fungi	131.4	356 0	837.0	413.0	363.0
	B + A	7.4	246.6	546.0	170.0	45.1
	A. per cent	32.0	1.9	1.0	2.5	5.7
4. Loamy garden soil, pH 8.03, + 1 per cent casein	Fungi	172.0	(0)	4.8	9.0	.....
	B + A	42.6	626.4	1,702.5	178.3	.....
	A per cent	19.3	6.7	0.4	3.6	.....
4a. Same + 2 per cent cellulose	Fungi	172.0	118.0	640.0	870.0	1,018.0
	B + A	42.6	1,798.0	448.0	78.0	105.7
	A. per cent	19.3	2.9	5.4	4.3	7.8

Penicillium spp. In the faintly acid soil no. 3 the multiplication of the fungi is less abundant, and the flora is mostly represented by *Mucor racemosus* and *Fusarium orthoceras*. In the alkaline soil no. 4 the fungi are nearly exterminated by the addition of casein alone; when cellulose too is added, there is a considerable multiplication of fungi, especially the strong cellulose decomposer *Coccospora agricola*, which is so markedly favored by alkaline reaction. The bacteria and actinomyces multiply enormously in the acid soil, but not so much

in the alkaline soils; this is probably because the ammonia production soon gives the acid soils a reaction favorable to the bacteria and actinomyces, but renders the alkaline soil too alkaline. Although thus the fungi are known to be most active in decomposing proteins in acid soils, they may be more or less active under alkaline conditions, especially when cellulosic material is also present, such as will mostly be the case under natural conditions. The Mucoraceae and Penicillia, and in some instances Fusaria, seem to be the most active fungi; *Trichoderma koningi*, which has been shown by several investigators to produce very large amounts of ammonia from proteins in pure cultures, does not appear to have been of any importance here, although it was present in the original fungus flora of all four soils.

TABLE 16

Multiplication of fungi, actinomyces, and bacteria in soils during decomposition of alfalfa seed meal

SOIL + 1 PER CENT ALFALFA SEED MEAL		START	AFTER 10 DAYS	AFTER 20 DAYS	AFTER 30 DAYS	AFTER 45 DAYS	AFTER 60 DAYS
1. Sandy forest soil, pH 4.54	Fungi*	247	199,200	72,600	59,400	48,300	56,800
	B + A†	2.5	12.4	16 0	8.4	4.1	3.5
	A. per cent	18.5	..... .	....	....	23.9	29.2
2. Heavy clay soil, no. 38, pH 4.96	Fungi	86	32,800	109,200	50,300	77,000	108,000
	B + A	1.9	45.0	....	62.0	26.8	17.4
	A. per cent	22.2	23.7	....	9.1	26.1	22.8
3. Light loam, pH 5.92	Fungi	131	6,430	5,140	5,300	4,430	5,860
4. Heavy clay soil, no. 38, + 1.0 per cent $CaCO_3$, pH after 10 days 7.48	Fungi	86	2,115	1,218	1,474	2,844	944
	B + A	1.9	230.0	280.7	223.4	217.6	162.0
	A. per cent	22.2	39.1	25.2	36.1	47.7	39.0
5. Loamy garden soil, pH 7.48	Fungi	192	2,135	1,280	1,020	646	350
	B + A	27.8	2,232.0	2,692.0	612.0	301.4	124.0
	A. per cent	24.4	26.3	29.3	21.1	33.6	44.0

* Fungi: 1,000 per gm. of soil.

† Bacteria + actinomyces: Millions per gm. of soil.

Decomposition of alfalfa seed meal

These experiments were carried out with the same soils and in the same manner as the previous set. The alfalfa seed meal contained 4.72 per cent nitrogen in air-dry matter and was added in a quantity of 1 per cent of air-dry soil. The results in table 16 are even more striking than those of the previous experiment.

In the strongly acid soils the multiplication of the fungi is so enormous that the mycelia for the first few weeks bind the soil together in one compact mass. The dominating species here are *Mucor ramannianus* and *Penicillium* sp. I and in no. 2, also *Mucor hiemalis*. The bacteria and actinomyces are only slightly affected. In the two alkaline soils, on the other hand, the development of the bacteria and actinomyces is enormous, and that of the fungi relatively scanty. Under these circumstances also they take an active part in the transformation of organic matter, as shown by their rising and then falling numbers and the still macroscopically visible development of mycelium. The fungus flora is here of another character than in the acid soils. It consists mainly of *Mucor hiemalis*, *Mucor racemosus*, and sterile Phycomycetous mycelium and, in the originally acid soil no. 4, also of *Penicillium* sp. and *Fusarium orthoceras*. The faintly acid soil represents an intermediate stage as to the multiplication of the fungi. Its flora consists of *Mucor racemosus*, *Mocor hiemalis*, *Penicillium* spp., and *Fusarium orthoceras*. The general aspect of the fungus flora is thus about the same as in the experiments with casein.

Proteolytic power of fungi in pure culture

To determine the comparative proteolytic powers of the various soil fungi, two sets of experiments were carried out.

Proteolytic activity in gelatin. In the first series of these experiments, fungi were grown as ordinary test tube cultures on slopes of 15 per cent "gold label" gelatin in distilled water, with and without 1 per cent dextrose. After 20 days' incubation at 25°C. the gelatin was melted, if not completely liquefied, and determinations were made, in 2-cc. portions, of ammonia-N by distillation with MgO, and of formol-titrating N by the method of Sørensen. The results are given in table 17.

In most cases the presence of dextrose decreases the extent of proteolysis, because the dextrose by acting as energy material saves the protein from attack (51). But it does not hold for the proteolytically strongest organisms, *Citromyces glaber*, *Fusarium orthoceras*, and two organisms included for comparison, viz., *Actinomyces griseus* and *Bacillus mycoides*. Their activity is in some cases actually increased by the presence of the sugar. Among the fungi only, *Fusarium orthoceras* is about equal to *Bac. mycoides* in proteolytic respect, and none of these reach *Actinomyces griseus*.

A somewhat different result was obtained in the second series of experiments. The following medium was used: gelatin, 20 gm.; dextrose (if added), 20 gm.; K_2HPO_4, 2 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.5 gm.; $FeCl_3$, 0.1 gm.; H_2O, 1,000 cc.; pH 6.4. The solution was used in 100-cc. portions in 350-cc. Erlenmeyer flasks. After incubation for 20 days at 25°C. the cultures were filtered, the mycelia dried and weighed, the filtrates restored to 100 cc., and determinations of ammonia-N and formol-titrating N carried out.

The results in table 18 show some interesting phenomena. In gelatine

solution without dextrose, *Citromyces glaber* is a fairly strong protein decomposer, and *Actinomyces griseus* a very strong one, whereas the four other fungi and *Bacillus mycoides* show very moderate activities. The growth of the fungi was in all cases poor and was limited to a loose, submerged mycelium without fructification. *Act. griseus* grew excellently, producing a heavy, firm, wrinkled pellicle, covered with a thick, dusty layer of conidia and emitting the strong characteristic soil odor. In the dextrose-cultures the activity of *Bac. mycoides* is strongly inhibited, probably because of acid-poisoning, since this organism forms acid from dextrose and had altered the reaction of the medium

TABLE 17

Proteolytic activity of fungi in 15 per cent gelatin with and without dextrose

ORGANISM	GELATIN WITHOUT DEXTROSE			GELATIN + DEXTROSE		
	Liquefaction	NH_3 – N, mgm. per 10 cc.	Formol-titrating N, mgm. per 10 cc.	Liquefaction	NH_3 – N, mgm. per 10 cc.	Formol-titrating N, mgm. in 10 cc.
Mucor hiemalis	Complete	14.2	16.1	About ¾	9.8	10.6
Mucor ramannianus	Very faint	4.2	2.7	None	1.7	1.4
Zygorhynchus sp.	About ½	15.4	15.4	About ½	....	7.1
Cephalosporium sp.	About ⅓	8.4	14.7	About ¾	5.6	6.3
Trichoderma koningi	Faint	7.0	9.8	Faint	....	7.0
Aspergillus fumigatus	Faint	9.1	11.2	Very faint	4.9	4.2
Penicillium sp. I	Faint	10.5	9.1	Faint	6.0	5.6
Citromyces sp. I	Complete	9.8	26.0	Nearly complete	14.0	25.9
Acrocylindrium sp.	About ⅓	9.5	14.7	About ¾	8.4	15.4
Coccospora agricola (?)	Complete	14.2	16.8	Complete	16.8	18.9
Mycogone nigra	Faint	11.9	9.8	About ⅓	5.3	2.1
Fusarium orthoceras	About ¾	21.7	27.3	Complete	33.6	48.3
Fusarium sp. II	About ¾	14.0	12.6	About ¾	16.5	11.9
Actinomyces griseus	Complete	23.8	51.8	Complete	45.5	54.6
Act. diastatechromogenus	About ⅓	11.2	37.1	About ¾	4.5	24.2
Bacillus mycoides	Complete	16.5	37.1	Complete	23.1	35.7
Control (sterile)	....	0.0	3.5	....	0.0	3.5

to pH 5.1. *Act. griseus* is hardly affected, but the fungi (except *Mucor ramannianus*, for which the reaction is probably the limiting factor) are greatly stimulated in their proteolytic activity, so much indeed, that three of them exceed *Act. griseus* in ammonia-formation and one in formation of formol-N. The explanation is probably that dextrose is so infinitely superior to gelatine as energy material for the fungi that their growth (except *Mucor ramannianus*) is greatly stimulated and appears as heavy, folded, abundantly sporulating mats, containing much more protoplasm than in the solution without gelatin, and this again gives rise to a production of much larger amounts of proteolytic

and de-amidizing enzymes. That the proteolytic activity of fungi is inhibited by the presence of non-nitrogenous energy material may thus be true only *relatively*, that is, when we compare equal amounts of synthesized mycelium, such as shown in columns 4 and 8 of table 18, where the amounts of formol-titrating N are taken as indexes of the proteolytic activity.

TABLE 18

Proteolytic activities of fungi in 2.0 per cent gelation solution with and without dextrose

ORGANISM	SOLUTION WITHOUT DEXTROSE				SOLUTION + DEXTROSE			
	NH_3-N, mgm. per 100 cc.	Formol-titrating N, mgm. per 100 cc.	Weight of dry mycelium, gm.	Formol-titrating N, mgm. per gm. of mycelium	NH_3-N, mgm. per 100 cc.	Formol-titrating N, mgm. per 100 cc.	Weight of dry mycelium, gm.	Formol-titrating N, mgm. per gm. of mycelium
Mucor ramannianus	15.3	21.0	(trace)	....	3.5	13.6	(trace)	....
Mucor racemosus	13.3	48.3	0.18	218	101.2	100.1	0 85	106
Penicillium sp. I	17.9	38.5	0.20	147	45.5	58.1	0.90	54
Citromyces glaber	67.9	101.1	0.18	505	148.4	144.9	0.65	207
Fusarium orthoceras	17.5	40.6	0.19	166	90.0	107.8	0 95	102
Actinomyces griseus	72.1	135.1	0.20	630	71.7	130.9	....	....
Bacillus mycoides	23.5	56.7	....	....	5.8	23.8		
Sterile solution	0.0	9.1	....	....	0.0	9.1	....	....

TABLE 19

Proteolytic activity of fungi in acid and neutral peptone solution

ORGANISM	ACID SOLUTION			NEUTRAL SOLUTION		
	NH_3-N, mgm. per 50 cc.	Formol-titrating N, mgm. per 50 cc.	pH	NH_3-N, mgm. per 50 cc.	Formol-titrating N, mgm. per 50 cc.	pH
Mucor hiemalis	21.8	23.0	....	14.4	22.3	....
Mucor ramannianus	13.7	30.4	7.43	6.1	18.5	7.26
Trichoderma koningi	16 0	22.7	7.72	10.1	15.7	8.30
Aspergillus niger	41.1	40.3	6.36	41.2	40.3	4.86
Penicillium sp. I	20.6	24.2	7.92	16.8	30.8	8.03
Citromyces sp. 1	49.1	49.9	....	49.1	50.7	....
Mycogone nigra	18.3	20.9	8.32	....	17.4	....
Fusarium orthoceras	19.6	20.2	8.8	16.8	20.2	9.0
Actinomyces griseus	No growth			13.5	34.5	9.0
Bacillus mycoides	16.4	9.0	6.85	18.5	28.6	8.14
Control (sterile)	1.1	6.2	5.03	1.1	6.5	7.0

Proteolytic activity in peptone. These experiments were carried out in solutions of acid and neutral reaction. The following medium was used: Peptone, 20.0 gm.; K_2HPO_4 or KH_2PO_4, 2.0 gm.; $MgSO_4$, 0.5 gm; NaCl, 0.5 gm; H_2O, 1000 cc. The reaction after sterilization was pH 4.9 with KH_2PO_4, pH 7.05 with K_2HPO_4. The solutions were used in portions of 20 cc. in 50-cc.

Erlenmeyer flasks. Cultures were incubated for 20 days at 25°C. and analysed as above. The results are found in table 19.

It is seen that the two strongest protein decomposers, *Citromyces glaber* and *Aspergillus niger*, are fairly indifferent toward changes in the reaction, whereas acid reaction is favorable to the rest of the fungi, especially *Mucor ramannianus*. Butkewitch (5) found that the proteolysis by fungi in acid solution is carried largely to the ammonia-stage. In the present experiment there is an indication of this only in the case of *Penicillium* sp. I. As might be expected, *Bact. mycoides* and *Act. griaseus* show only slight or no activity in acid solution, and even in neutral solution their activity is not greater than that of several fungi.

These experiments indicate that the extent of proteolysis by fungi is very variable and depends, among other things, on the nature of the protein, the presence of carbonaceous food, and the reaction of the medium, but is often comparable to or may exceed that of *Bac. mycoides* and *Act. griseus*—two soil organisms known to possess particularly strong proteolytic properties.

It might now be asked whether any relation exists between the proteolytic powers of the fungi in pure culture and their activity in protein decomposition in the soil, as indicated by their multiplication. It will be noted that two of the strongest protein decomposers—*Asp. niger* and *Citr. glaber*—appear to play no rôle, since the former does not occur in normal soils, and the latter, although of common occurrence, is not prevalent in the flora developing during the decomposition of casein or alfalfa seed meal in the soil. This is the case with *Mucor hiemalis*, *Mucor racemosus*, *Penicillium* sp. I, and *Fusarium orthoceras*, of which especially the last has shown strong proteolytic properties. A special position is occupied by *Mucor ramannianus*, which multiplies vigorously in acid soil during decomposition of protein and is capable of exerting strong proteolytic activities in acid media, but is greatly inhibited by neutral or alkaline reaction.

GENERAL CONCLUSIONS

The experiments with addition of organic matter to the soil show better than the counts from soils in their natural state the influence of soil reaction on the microflora. Whereas the plate counts show that the ratio of fungi to bacteria plus actinomyces is governed principally by the hydrogen-ion concentration, which exerts its most pronounced influence in the pH interval from 5 to 6, the decomposition experiments show that the presence of decomposable organic material tends to stimulate the activities of fungi under acid and, to a lesser extent, alkaline conditions. Here again, the change in the preponderance of fungi over bacteria plus actinomyces, and vice versa, is marked in the pH interval from 5 to 6, as is shown by the experiments with dextrose, casein, and alfalfa seed meal. This change is hardly due to any specific sensitiveness of most soil fungi to alkalinity, but rather to the sensitiveness of most soil bacteria and actinomyces to acidity. It is probably from this stimulating effect

of organic matter on both the main groups of soil microörganisms, that the positive correlation between numbers of fungi and bacteria plus actinomyces results. This effect depends not only on the hydrogen-ion concentration, but also on the quality of the organic compounds, of which dextrose and casein stimulated only the bacteria in the alkaline soil, whereas the cellulose stimulated the fungi markedly and had but a slight and probably only secondary effect on the bacteria and actinomyces developing in plate counts. The fact that there is a definite correlation between the numbers of fungi and the numbers of bacteria plus actinomyces in soils where the influence of the reaction is no longer noticeable, suggests that the number of fungi in the soil obtained by the plate method is not merely a chance figure dependent on the accidental number of fungus spores, but has a real bearing on the abundance of fungi in the soil, although, as has been pointed out, the figure refers to the soil suspension and not to the soil itself. Also we must remember that in several of the decomposition experiments, such as those with cellulose and alfalfa seed meal in acid soil, there was in the later stages of the process an abundant, macroscopically visible fructification of the fungi. This is reflected in the constantly high counts of fungi in such cases (in opposition to those where the fungus counts, after having reached a maximum, soon decrease again, for instance with casein in acid soil and alfalfa seed meal in alkaline soil). In such cases the fungus counts become counts of spores and loose all significance as indexes of activity of the fungi, although of course the rise in numbers indicates that an active growth has taken place. In future work of this nature it would probably prove of great value to combine decomposition experiments with the method of McLennan (32) for distinguishing between fungus spores and mycelium in the soil.

SUMMARY

A study has been made of the fungus flora of 100 Danish soils of different types and of reactions varying from pH 3.34 to 8.35.

Microscopical examination showed the largest amounts of mycelium in acid soils rich in organic matter.

"Direct isolation" yielded mainly Trichodermae from forest, moor, and heath soils, and mainly Mucoraceae from field, garden, and salt marsh soils.

Plate counts showed numbers of fungi ranging from 24,300 to 46,000 to a gram of soil. Most common were the genera *Mucor*, *Zygorhynchus*, *Absidia*, a sterile form resembling *Cunninghamella elegans*, *Penicillium*, *Trichoderma*, and *Fusarium*. Besides these a number of genera of less constant occurrence were found. The Aspergilli were found only sporadically in ordinary soils but very abundantly in hot greenhouse soils. The genera *Fusarium* and *Phoma* were characteristic of cultivated soils.

The numbers of fungi showed no clear relationship to the type of soil, except that very heavy clay soils were poor in fungi; and no relation to the soil reaction was found. The numbers of bacteria plus actinomyces increased, generally,

with increasing pH values up to pH above 6, but the correlation between these numbers and the hydrogen-ion concentration was not very close, the correlation coefficient amounting to only −0.32. The ratio of fungi to bacteria plus actinomyces varied from 0.87 to 0.0026 and showed a very close correlation to the hydrogen-ion concentration, viz., a correlation coefficient of 0.82 for the whole set of data. From pH 6 and upwards this correlation did not exist, but it became manifest in the pH interval 5–6. A corresponding coefficient of very nearly the same numerical value could be calculated from the data of Waksman.

Addition of lime to acid soils did not markedly depress the numbers of fungi, but stimulated the bacteria and actinomyces greatly.

The resistance of fungi to acidity varies considerably, some species growing at pH 1.5, others being checked at pH 3.7–4.2. Only a few of them show a distinct optimum at acid reaction. The pH interval of 5.0–6.0 is critical for the majority of the ordinary soil bacteria, and most soil actinomyces are checked in their growth at pH 4.4–5.

Fertilization, especially with farmyard manure, increases the number of fungi in the soil, as well as those of bacteria and actinomyces. In soils of pH above 6 there is a significant positive correlation between the numbers of fungi and of bacteria plus actinomyces.

These results indicate that the actual abundance of fungi depends on many factors, among others the food supply, but the ratio of fungi to bacteria plus actinomyces seems dependent on little except the hydrogen-ion concentration.

Addition of dextrose to soil stimulated the fungi greatly in acid soil, but little or not at all in neutral or alkaline soil. The bacteria were affected reversely. Also here the difference appeared in the pH interval 5.1 to 5.9. The sugar appeared to be equally rapidly decomposed in acid and alkaline soil.

Most fungi except the Mucoraceae were capable of decomposing cellulose. Addition of cellulose gave rise to an abundant development of fungi in both acid and alkaline soil. Penicillia and Trichodermae prevailed in the acid soil, *Mycogone nigra* and *Coccospora agricola* in the alkaline soil. The fungi of the former group were more resistant to acidity and decomposed cellulose more rapidly in acid soil, whereas the reverse was true of those of the latter group.

Addition of casein gave rise to an abundant development of fungi (especially of Penicillia and Mucoraceae) in acid soil but not in alkaline soil, except when cellulose was also added.

Alfalfa seed meal caused an abundant development of Penicillia and Mucoraceae in acid soil, and a somewhat more limited growth of Mucoraceae and Fusaria in neutral to alkaline soil.

The proteolytic power of the soil fungi varied considerably according to the experimental conditions, but would in several instances rival or exceed that of *Bacillus mycoides* and *Actinomyces griseus*. The most strongly proteolytic fungi were not necessarily those appearing to grow most actively when protein was added to the soil.

REFERENCES

(1) Appel O, and Wollenweber, H. W. 1910 Grundlagen einer Monographie der Gattung *Fusarium* (Link). *Arb. K. Gsndhtsamt. Biol. Abt.* 8: 1–156.

(2) Barthel, C., and Bengtsson, N. 1924 Action of stable manure in the decomposition of cellulose in tilled soil. *Soil Sci.* 18: 185-216.

(3) Brierley, W. B. 1923 Soil fungi (in *Microorganisms of the soil* by E. J. Russell, Longman's Green & Co., New York).

(4) Brierley, W. B., Jewson, S. T., and Brierley, M. 1928 The quantitative study of soil fungi. *Proc. First Internatl. Cong. Soil Sci.* 3: 48-71.

(5) Butkewitch, W. 1903 Umwandlung der Eiweissstoffe durch die niederen Pilze. *Jahrb. Wiss. Bot.* 38: 147–240.

(6) Charpentier, G. A. C. 1921 Studien über den Einfluss des Rindvieh- und Pferdestallmistes auf die Zersetzung der Zellulose in der Ackererde. Thesis, Tavastehus.

(7) Christensen, H. R. 1927 Jordbvundsundersøgelser i Forbindelse med fastliggende Gødningsforsøg paa Mineral jorder. *Tidsskr. Planteavl.* 33: 197-411.

(8) Collman, D. A. 1916 Environmental factors influencing the activity of soil fungi. *Soil Sci.* 2: 1–66.

(9) Conn, H. J. 1922 A microscopic method for demonstrating fungi and actinomycetes in the soil. *Soil Sci.* 14: 149–151.

(10) Currie, J. 1917 The citric acid fermentation of *Aspergillus niger*. *Jour. Biol. Chem.* 31: 15.

(11) Daszewska, W. 1913 Étude sur la désagregation de la cellulose dans la terre de Bruyère et la Tourbe. *Bull. Univ. de Genève, Inst. Bot.* 8: 238 316.

(12) Dubos, R. J. 1928 Influence of environmental conditions on the activities of cellulose decomposing bacteria in the soil. *Ecology* 9: 12–27.

(13) Dubos, R. J. 1928 The decomposition of cellulose by aerobic bacteria, *Jour. Bact.* 15: 223–234.

(14) Fischer, H. 1909 Über den Einfluss des Kalkes auf die Bakterien eines Bodens. *Landw. Vers. Stat.* 70: 335.

(15) Fischer, H. 1909 Bakteriologisch-Chemische Untersuchungen. Bakteriologischer Teil. *Landw. Jahrb.* 38: 355 264.

(16) Fisher, R. A. 1925 Statistical Methods for Research Workers. London and Edinburgh.

(17) Goddard, H. N. 1913 Can fungi living in agricultural soil assimilate free nitrogen? *Bot. Gaz.* 56: 249 305.

(18) Hagem, O. 1907 Untersuchungen über norwegische Mucorineen. I *Vidensk.-Selsk, Skr. (Christiania). Mathem.-Naturv. Kl.* 7: 1 50.

(19) Hagem, O. 1910 Untersuchungen über norwegische Mucorineen. II *Vidensk.-Selsk. Skrifter* (Christiania). *Math.-Naturv. Kl.* 10: 1 152.

(20) Hopkins, E. F. 1921 Growth and germination of *Gibberella saubinetti* at varying hydrogen-ion concentrations. *Phytopathology* 11: 36.

(21) Itano, A. 1925 Biological investigation of peat. *Jour. Bact.* 10: 87 91.

(22) Iterson, C. v. 1904 Die Zersetzung von Cellulose durch aerobe Mikroorganismen. *Centbl. Bakt.* (II) 11: 689–698.

(23) Jensen, C. N. 1912 Fungus flora of the soil. Cornell (N. Y.) Agr. Exp. Sta. Bul. 315.

(24) Jensen, H. L. 1930 Some observations on actinomycetes in Danish soils. *Soil Sci.* 30: 59–77.

(25) Johnson, H. W. 1923 Relationship between hydrogen ion, hydroxyl ion, and salt concentrations on the growth of seven soil molds. Iowa Agr. Exp. Sta. Res. Bul. 76.

(26) Koning, C. J. 1904 Bijdrage tot de Kenntnis van het Leven der humicole Fungi (etc.) Verhandel. K. Akad. Wetensch. Amsterdam, 2. Sect. 9, no. 7.

(27) Kopeloff, N. 1916 The effect of soil reaction on the ammonification by certain soil fungi. *Soil Sci.* 1: 541–574.

(28) Lendner, A. 1908 Les Mucorinees de la Suisse. Berne.

(29) Lundegårdh, H. 1923 Die Bedeutung des Kohlensäuregehaltes und der Wasserstoffionenkonzentration des Bodens für die Entstehung der Fusariosen. *Bot. Notiser* 1923: 25.

(30) McBeth, J. C., and Scales, F. M. 1913 The destruction of cellulose by bacteria and filamentous fungi. U. S. Dept. Agr. Bur. Plant Indus. Bul. 266.

(31) MacInnes, J. 1922 The growth of the wheat scab organism in relation to hydrogen-ion concentration. *Phytopathology* 12: 290–294.

(32) McLennan, E. 1928 The growth of fungi in the soil. *Ann. Appl. Biol.* 15: 95–109.

(33) Marchal, E. 1893 Sur la production de l'ammoniaque dans le sol par les microbes. *Bul. Acad. Roy. Sci. Belg.* 25: 727–771.

(34) Meacham, M. R. 1918 Note upon the hydrogen-ion concentration necessary to inhibit the growth of four wood-destroying fungi. *Science* 48: 499.

(35) Melin, E. 1924 Über den Einfluss der Wasserstoffionenkoncentration auf die Virulenz der Wurzelpilze von Kiefer und Fichte. *Bot. Notiser.* 1924: 38.

(36) Müller, P. E. 1883 Studer over Skovjord. Copenhagen.

(37) Müller, P. E., Rordam, K., Helms, J., and Woldike, E. H. 1910 Bidrag til Kundskab om Rødgranens Vaekstforhold i midtjydsk Hedebund. *Forstl. Forsøgsv. Danmark* III: 1–270.

(38) Müntz, A., and Coudon, H. 1893 La fermentation ammoniacale de la terre. *Compt. Rend. Acad. Sci (Paris)* 116: 395–398.

(39) Otto, R. 1916 Untersuchungen über die Auflösung von Zellulosen und Zellwänden durch Pilze. *Beitr. Allg. Bot.* 1: 190–260.

(40) Petersen, E. J. 1926 Undersøgelser over Forholdet mellem Jordens Kulsyreproduktion, kemiske Tilstandsform og mikrobiologiske Aktivitet. *Tidsskr. Planteavl.* 32: 625–672.

(41) Ramann, E., Remele, C., Schellhorn, and Krause, M. 1899 Anzahl und Bedeutung der niederen pflanzlichen Organismen in Wald- und Moorböden. *Ztschr. Forst. u. Jagdw.* 31: 575–606.

(42) Rege, R. D. 1927 Biochemical decomposition of cellulosic materials with special reference to the action of fungi. *Ann. Appl. Biol.* 14: 1–44.

(43) Rostrup, O. 1916 Bidrag til Danmarks Svampeflora I. *Dansk Bot. Ark.* 2: no. 5.

(44) Sopp, O. J. -O. 1912 Monographie der Pilzgruppe *Penicillium*. *Vidensk. -Selsk. Skr. (Christiania), Mathem. -Naturv. Kl.* No. 11.

(45) Starkey, R. L. 1929 Some influences of the development of higher plants upon the microörganisms in the soil: II. Influences of the stage of plant growth upon abundance of organisms. *Soil Sci.* 27: 355–378.

(46) Starkey, R. L., and Henrici, A. T. 1927 The occurrence of yeasts in soil. *Soil Sci.* 23: 33–44.

(47) Traaen, A. E. 1914 Untersuchungen über Bodenpilze aus Norwegen. *Nyt Mag. Naturv.* 52: 20–121.

(48) Waksman, S. A. 1916 Soil fungi and their activities. *Soil Sci.* 2: 103–156.

(49) Waksman, S. A. 1916 Do fungi actually live in the soil and produce mycelium? *Science* 44: 320–322.

(50) Waksman, S. A. 1917 Is there any fungus flora of the soil? *Soil Sci.* 3: 565–589.

(51) Waksman, S. A. 1918 Studies on proteolytic activities of soil microörganisms with special reference to fungi. *Jour. Bact.* 3: 475–492.

(52) Waksman, S. A. 1922 A method for counting the numbers of fungi in the soil. *Jour. Bact.* 7: 339–341.

(53) Waksman, S. A. 1922 Microbiological analysis of soil as an index of soil fertility: II. Methods for the study of numbers of microorganisms in the soil. *Soil Sci.* 14: 285–298.

(54) WAKSMAN, S. A. 1922 Microbiological analysis of soil as an index of soil fertility: III. Influence of fertilization upon numbers of microorganisms in the soil. *Soil Sci.* 14: 321–346.

(55) WAKSMAN, S. A. 1922 The influence of the soil reaction on the growth of actinomycetes causing potato scab. *Soil Sci.* 14: 61–79.

(56) WAKSMAN, S. A. 1924 Influence of microorganisms upon the carbon-nitrogen ratio in the soil. *Jour. Agr. Sci.* 14: 555–562.

(57) WAKSMAN, S. A. 1927 Cellulose als eine Quelle des "Humus" im Erdboden. *Cellulosechemie* 8: 97–103.

(58) WAKSMAN, S. A. 1927 Principles of Soil Microbiology. Baltimore.

(59) WAKSMAN, S. A., AND JOFFE, J. S. 1920 Studies in the metabolism of actinomycetes: IV. Changes in the reaction as a result of the growth of actinomycetes upon culture media. *Jour. Bact.* 5: 31–48.

(60) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Influence of organic matter upon the development of fungi, bacteria, and actinomycetes in the soil. *Soil Sci.* 17: 373–378.

(61) WAKSMAN, S. A., AND HEUKELEKIAN, O. 1924 Microbiological analysis of soil as an index of soil fertility: VIII. Decomposition of cellulose. *Soil Sci.* 17: 275–291.

(62) WAKSMAN, S. A., AND LOMANITZ, S 1925 Contribution to the chemistry of decomposition of proteins and amino acids by various groups of microorganisms. *Jour. Agr. Res.* 30: 263–281.

(63) WAKSMAN, S. A., AND HEUKELEKIAN, O. 1926 Cellulose decomposition by various groups of soil microorganisms. *Actes IV-ieme Conf. Internatl. Pedol. Rome 1924*, III: 216–228.

(64) WAKSMAN, S. A., AND SKINNER, C. E. 1926 Microorganisms concerned in the decomposition of celluloses in the soil. *Jour. Bact.* 12: 57–84.

(65) WAKSMAN, S. A., AND STEVENS, K. R. 1929 Contribution to the chemical composition of peat: V. The rôle of microörganisms in peat formation and decomposition. *Soil Sci.* 27: 315–340.

(66) WEIS, F., AND BONDORFF, K. A. 1917 Kemisk-biologiske Undersøgelser af Skovjord under "overernaerede Graner" paa Wedellsborg Skovdistrikt. *Dansk Skovforen. Tidsskr.* 1917: 143–153.

(67) WEIS, F., AND NIELSEN, N. 1927 Nogle Undersøgelser over Rodfordaerversvampen (*Polyporus radiciperda*). *Meddelel. Dansk Skovforening* (etc.) V: 235–246.

(68) WERKENTHIN, F. C. 1916 Fungus flora of Texas soils. *Phytopathology* 6: 241–253.

(69) WINOGRADSKY, S. 1924 La methode directe dans l'Etude microbiologique du Sol. *Chim. et Indus* (Paris) 11: 215–222

THE NATURE OF SOIL ACIDITY AS AFFECTED BY THE SiO_2-SESQUIOXIDE RATIO[1]

L. D. BAVER AND G. D. SCARSETH[2]

Alabama Agricultural Experiment Station

Received for publication April 28, 1930

The modern trend of thought regarding the nature of base exchange reactions has rather definitely related soil acidity to the absorptive properties of the soil. There have been many different views presented, however, to explain the exact relation between these exchange properties and soil acidity. Although the investigations of Bradfield, Kelley, Mattson, and Truog in this country and the work of the Europeans, Gedroiz, Hissink, Odén, Page, and others, have solved a number of problems related to soil acidity, many conflicting ideas that need to be clarified have arisen from their studies.

It is the purpose of this paper to show the differences in the acids from various soils and to discuss the relationships existing between these acids and the kind and extent of weathering under which the soil has developed; to show the relation of the nature of the soil acids to the chemical composition of the soil colloidal material as expressed by the SiO_2-sesquioxide ratio; and to discuss the reliability of the methods for determining the chemical composition and nature of the soil exchange complex.

REVIEW OF LITERATURE

Most soil investigators agree that base exchange and soil acidity phenomena originate in the complex ferro- and alumino-silicates found in the soil colloidal material. Bradfield (4) in his original work on the chemical reactions of a colloidal clay, advanced the theory that the colloidal clay of an acid soil is a true acid which ionizes to give a definite H-ion concentration. He considered these colloidal particles as large complex ions. The titration curve of the soil acid showed the characteristic properties of a monobasic acid. It was well buffered throughout the entire pH range. Although it acted similarly to a monobasic acid, Bradfield considers its ionization as representing a series of mass action relations as expressed by the equation:

$$\frac{[H^+]\,[X^-]}{[HX]_n} = K_a,\ K_b,\ K_c \text{———} K_n$$

where K_a, K_b....K_n represent the dissociation constants of a series of acids. The explanation for obtaining the typical monobasic acid titration curve in lieu of this series of acids is based upon the fact that a mixture of acids with unlike dissociation constants may give the

[1] Published with the approval of the director. Paper read before the division of agricultural and food chemistry at the Atlanta meeting of the American Chemical Society.

[2] Associate and assistant soil chemist, respectively.

typical monobasic curve if their respective constants are close enough together so that their individual curves overlap. The relative amount of any one acid would probably determine the exact shape of the curve. One of the writers (3) has shown that the nature of the titration curve or any particular soil acid is dependent upon the valance and hydration of the cation of the base with which the acid is titrated. Further work by Bradfield (5) has confirmed his original theory that soil acidity is due to complex colloidal alumino-silicic acids that dissociate in a similar manner to ordinary monobasic acids. Recent work by one of the writers (3) has shown that the physico-chemical properties of the soil colloidal material are functions of the degree of dissociation of these complex colloidal acids and their salts.

Kelley and Brown (11), from an analysis of their work on base exchange, advance a similar explanation for soil acidity, stating that the H-ion concentration of the soil is determined by the products of dissociation of the exchange complex, the hydrolytic products of the complex, and the buffer properties of the soil. They consider the replaceable bases in a soil as being the cations of salts of one or more alumino-silicic acids. Page (17) states that all soil acidity is essentialy of one kind, namely, that it is due to exchangeable hydrogen. The absorbing complex of the soil is considered as being an "acidoid," an insoluble colloidal acid, which is associated with surface active hydrogen and basic cations. For any given soil this complex possesses a fairly well-defined saturation capacity. Odén (16) accepts the views of Page and Bradfield, stating that these "acidoid" elements are a result of the weathering processes and that each soil will have a relatively constant pH due to the relatively constant rainfall and the composition of the mineral part of the soil. The fixation of H ions is attributed to the SiO_2 in the exchange complex, and the fixation of OH ions to the Al_2O_3 and Fe_2O_3.

Kerr (12, 13) has shown that the exchange complex is composed of definite chemical compounds having a characteristic crystalline form. He comes to the same conclusion as Bradfield and others that base exchange and soil acidity are due to complex alumino-silicates. He further concludes, however, that the predominant, if not the only, mineral in the inorganic exchange complex is a monobasic alumino-silicic acid corresponding to the formula

$$H_2O\ (Al_2O_3 \cdot 6\ SiO_2)\ 8H_2O$$

Irrespective of the nature of weathering processes, this compound is considered as being the sole acid that imparts acidity to a soil. The difference between the acidity of different soils is one of degree and not of kind. Joffe and McLean (10) suggest that various soils possess the same type of colloidal substance that varies only in its degree of dispersion. The results in this paper will aid in clarifying these divergent opinions.

The chemical composition of the soil colloidal material as expressed by the SiO_2-sesquioxide ratio varies considerably in different soils. Robinson and Holmes (20) have reported the results of numerous analyses of soil colloids which indicate that the extent of leaching of various soils is responsible for these variations in composition. Since the soil colloidal material is chiefly composed of the products of weathering of various soil-forming minerals, its composition varies considerably depending upon the kind and extent of weathering. Colloidal material extracted from soils developed under humid conditions and fairly high temperatures tend to have a low SiO_2-sesquioxide ratio because the silica is removed from the soil by the weathering processes. On the other hand, the colloidal material from soils weathered in a humid climate with moderately low temperatures tends to have a high ratio, because aluminum and iron are leached away. Therefore, the soils from the north (podzolic soils) have a higher ratio than those weathered under tropical or subtropical conditions (lateritic soils). Gile (9) states that different soil colloidal materials are of the same general nature, varying only in their SiO_2-sesquioxide ratio. The nature of the colloid is changed by varying the content and nature of the silica.

Numerous investigators have shown that the properties of the soil colloidal material vary with the SiO_2-sesquioxide ratio. Anderson and Mattson (2) have shown that the heat of wetting, and the absorptive capacity of the soil for NH_3 and malachite green are proportional

TABLE 1

Description of soils used in this study

SOIL NUMBER	SOIL TYPE	LOCATION	DEPTH	REMARKS
			inches	
386	Decatur silt loam	Alabama	0–6	Highly weathered soil from an old limestone valley
557	Lintonia silt loam	Arkansas	0–6	River terrace soil, moderately weathered
671	Cory silt loam	Illinois	0–6	Highly weathered soil from the Illinoian drift
674	Cecil clay loam	Alabama	0–6	Highly weathered soil from silicate rocks in the Piedmont Plateau
675	Norfolk sandy loam	Alabama	0–6	Soil weathered from unconsolidated sandstone in the Coastal Plain, not weathered as highly as soils 386 and 674
676	Colby silt loam	Wisconsin	0–6	Loessial soil associated with late Wisconsin Drift, moderately weathered
679	Miami silt loam	Wisconsin	0–6	Moderately weathered limestone soil from late Wisconsin Drift
680	Greenville fine sandy loam	Alabama	0–6	Soil from the Coastal Plain of limestone origin, weathered in a similar manner to soil 675
707A*	Susquehanna clay	Alabama	0–8	Grayish-brown, loamy fine sand
707B	Susquehanna clay	Alabama	8–20	Bright red, well-oxidized clay
707C	Susquehanna clay	Alabama	20–30	Sticky, heavy, blue-mottled, red clay
707D	Susquehanna clay	Alabama	At 10 feet	Blue clay, slightly mottled with yellow
710A†	Oktibbeha clay	Alabama	0–2	Brown clay loam
710B	Oktibbeha clay	Alabama	2–6	Yellow-brown clay
710C	Oktibbeha clay	Alabama	6–12	Reddish-brown, mottled with yellow, heavy clay
710D	Oktibbeha clay	Alabama	12–18	Yellow, mottled with red, heavy clay
722A†	Eutaw clay	Alabama	0–4	Light brown clay
722B	Eutaw clay	Alabama	4–14	Yellow, mottled olive-green and gray, sticky clay
742A†	Lufkin clay	Alabama	0–6	Gray-brown, mottled clay
742B	Lufkin clay	Alabama	6–15	Sticky, gray clay
743	Putnam clay	Missouri	14–24	Layer of accumulation of the Putnam silt loam, well-weathered prairie soil

* The Susquehanna clay occupies the hills and rolling areas on the inner border of the Coastal Plain, originating from clay deposits of marine origin. It is moderately weathered in the surface horizons.

† The Lufkin, Eutaw, and Oktibbeha clays represent soils from the coastal plain Black Belt. They are of marine origin and have been deposited upon a bed of limestone known as the Selma chalk. Their extremely impervious nature has hindered the weathering processes. The Lufkin is the most highly unweathered of these soils. The Oktibbeha is weathered the most. The Eutaw occupies an intermediary position in regards to weathering.

to this ratio. Parker and Pate (18) report that there is a good correlation between the SiO_2-sesquioxide ratio of the soil colloids and their exchangeable base content. Colloids with a high ratio tend to have more exchangeable bases. Mattson (15) has fairly definitely established that the capacity of the exchange complex to absorb cations increases with this ratio. The absorptive capacity of the soil for cations increases after the removal of the free sesquioxides. Adsorption of anions increases with a decrease in the ratio. He attributes the absorption of anions to the free sesquioxides that the soil may contain. Anderson also states that the absorptive capacity and exchangeable base content of soils are dependent upon this ratio. Although these data do not show an exact relationship between the base exchange properties of the soil colloids and the SiO_2-sesquioxide ratio, undoubtedly the absorptive properties of soil colloids are dependent in some way upon this ratio.

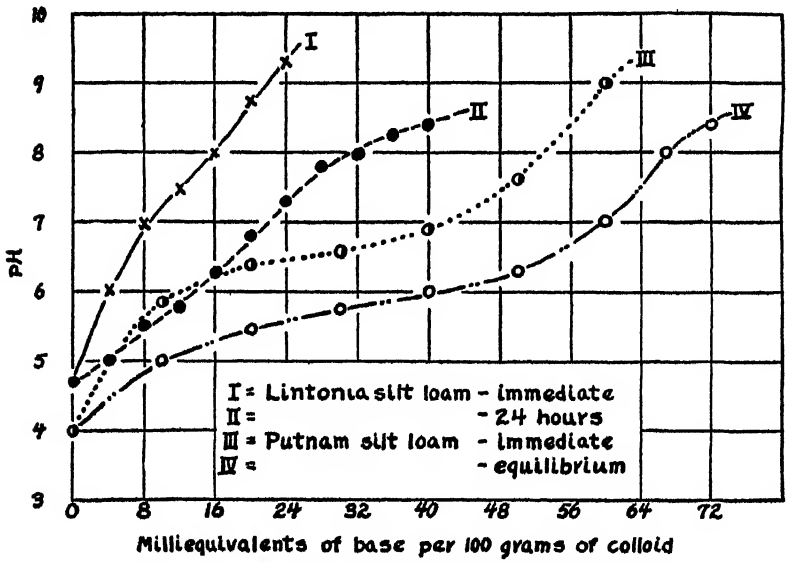


FIG. 1. THE EFFECT OF TIME ON THE TITRATION CURVES OF COLLOIDAL CLAY ACIDS

EXPERIMENTAL

Colloidal material extracted from 27 different soils, by the method of Bradfield (4), was used in this investigation. Since several of these soils were of the same soil series and type, the results on only 21 soils are reported in this paper. The curves for soils from the same series were practically identical in nature In order to show more clearly the wide differences in these soils with respect to origin and the kind and extent of weathering, a brief description is given in table 1.

Two grams of colloidal material extracted from each of these soils were electrodialyzed in a Bradfield three-compartment cell until all of the exchangeable cations were removed. The contents of the cathode chamber were analyzed for exchangeable cations. The electrodialyzed colloids were transferred from the cell to Erlenmeyer flasks. A 1 per cent colloidal suspension was obtained after the cell was rinsed out. Then suspensions were titrated electrometrically

with 0.1 N NaOH, the hydrogen electrode being used. The suspensions were shaken on a mechanical shaker for 2 hours after the addition of the base and allowed to stand for approximately 18 hours in order to attain equilibrium. Readings taken immediately after the addition of NaOH were found to be unreliable because equilibrium between the base and the acid clay had not been obtained. This is clearly shown in figure 1. Bradfield (6) has shown that this method of removing all of the exchangeable bases from soil colloids and titrating the resulting H-colloid with a base not only gives the total exchange capacity

TABLE 2

Soil acidity data on the soils used in this investigation

SOIL NUMBER	SOIL TYPE	$\frac{SiO_2}{R_2O_3}$	TOTAL EXCHANGE CAPACITY M.E. PER 100 GM. COLLOID	pK OF SOIL ACID	DEGREE OF DISSOCIATION OF SOIL ACID*	EXCHANGE CAPACITY ÷ pK VALUE
					per cent	
386	Decatur silt loam	1.73	11	6.15	23.0	1.80
557	Lintonia silt loam	2.60	22	5.75	4.5	3.82
671	Cory silt loam	3.20	22	5.65	40.4	3.90
674	Cecil clay loam	1.60	13	6 45	6.1	2.00
675	Norfolk sandy loam	1.92	22	6.85	8.1	3.20
676	Colby silt loam	3.18	32	6.10	8.8	5.25
679	Miami silt loam	3.97	30	6.25	11.8	4.80
680	Greenville fine sandy loam	1.62	20	6.50	8.8	3.10
707A	Susquehanna clay	2.27	28	5.85	25.2	4.80
707B	Susquehanna clay	2.11	36	6.35	14.0	5.70
707C	Susquehanna clay	3.25	55	6.25	8.1	8.80
707D	Susquehanna clay	3.50	46	5.20	7.8	8.80
710A	Oktibbeha clay	1.81	34	5.20	41.4	6.50
710B	Oktibbeha clay	1.83	34	5.75	46.4	5.90
710C	Oktibbeha clay	1.90	22	6.05	7.2	3.65
710D	Oktibbeha clay	1.92	22	6.30	6.4	3.50
722A	Eutaw clay	2.18	27	5.55	11.7	4.70
722B	Eutaw clay	2.31	33	5.15	15.1	6.40
742A	Lufkin clay	3.85	30	6.00	11.8	5.00
742B	Lufkin clay	3.81	30	5.70	16.6	5.30
743	Putnam clay	3.20	57	5.75	22.0	9.90

* Determined at the pH of the pure acid colloidal clay.

of the colloid, but also determined the dissociation constant of the soil acid.

The SiO_2-sesquioxide ratio was determined in the usual way. TiO_2 was not included with the Al_2O_3.

RESULTS

The titration curves of these soil acids are graphically shown in figures 2, 3, 4, 5, and 6. The SiO_2-sesquioxide ratio, exchange capacity, pK values and the degree of dissociation of the acids are given in table 2. These curves show

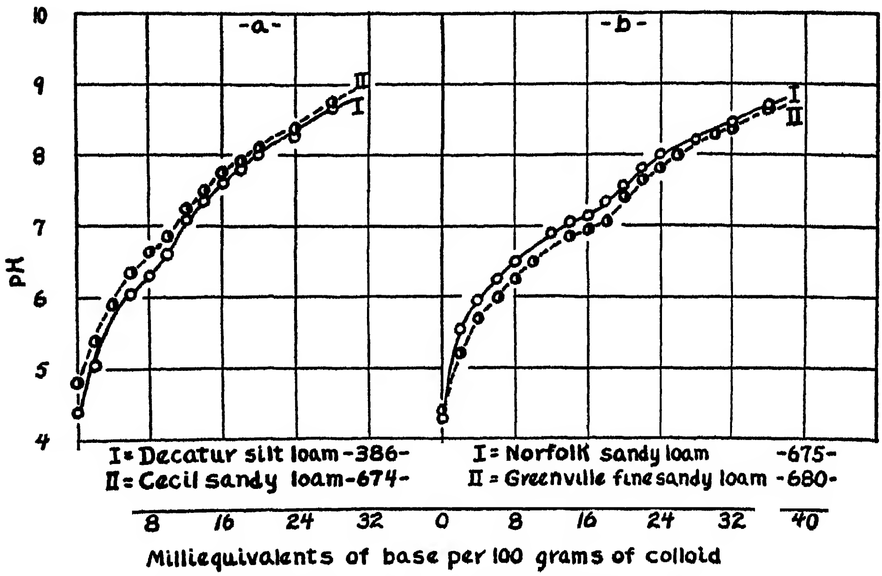


FIG. 2. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM VARIOUS SOUTHERN SOILS

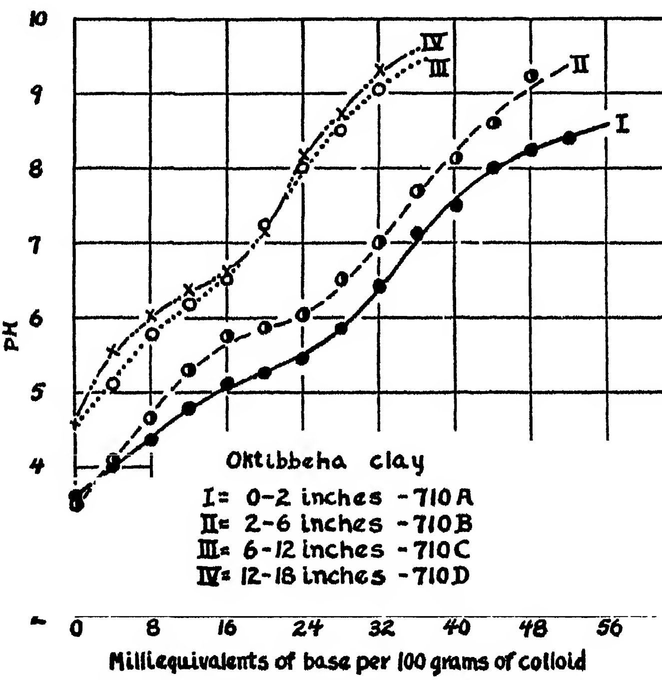


FIG. 3. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM OKTIBBEHA CLAY

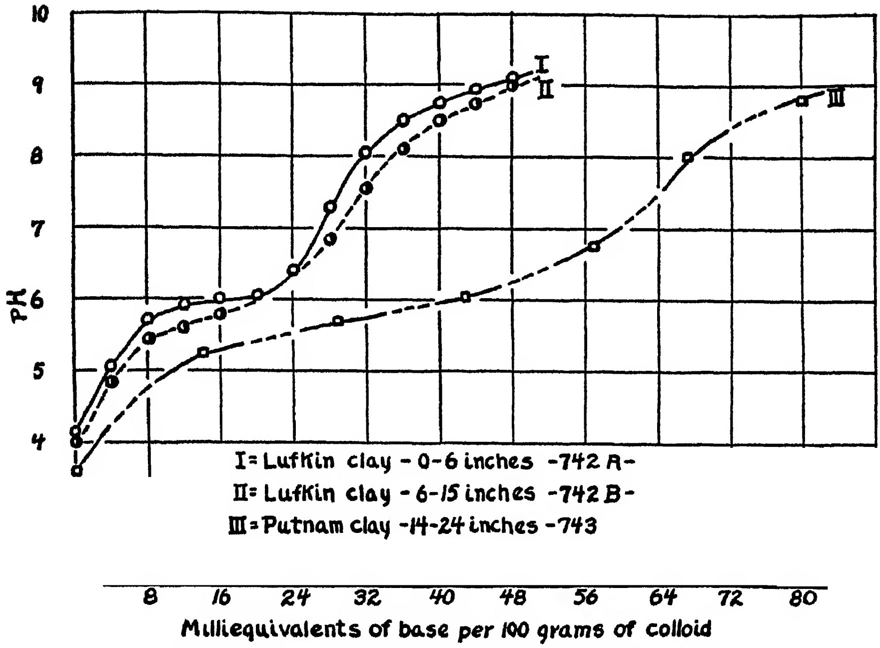


FIG. 4. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM DIFFERENT CLAYS

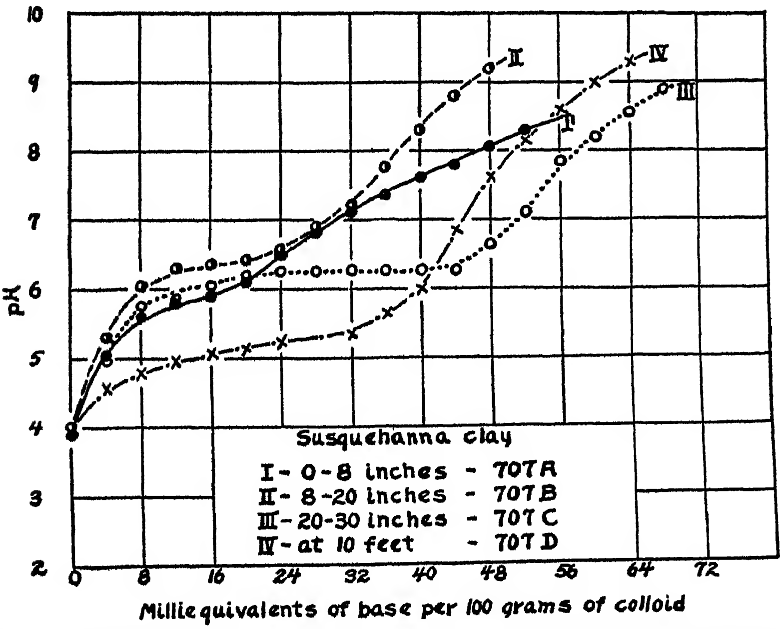


FIG. 5. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM DIFFERENT HORIZONS OF SUSQUEHANNA CLAY

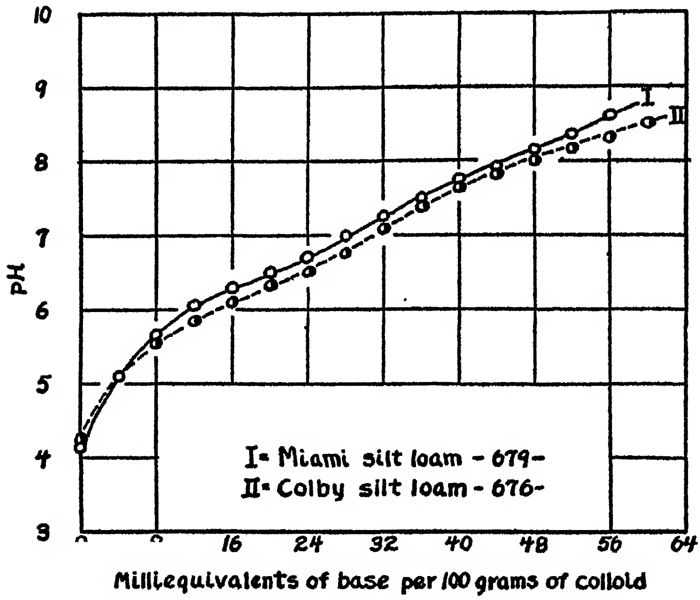


FIG. 6. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM VARIOUS SILT LOAMS

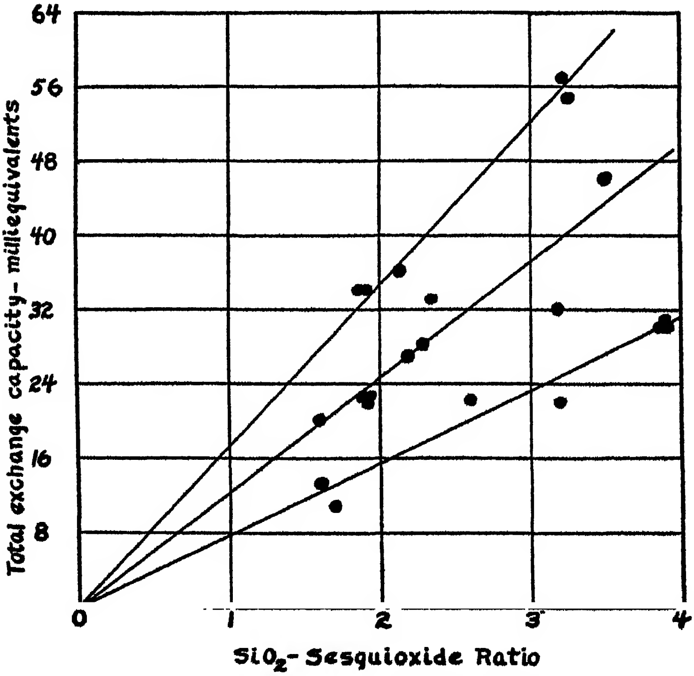


FIG. 7. THE RELATION OF THE TOTAL EXCHANGE CAPACITY TO THE SiO_2-SESQUIOXIDE RATIO

very clearly that the nature of the soil acid in all of these soils is not the same. Although all of the curves have the same general shape, similar to a monobasic acid, their respective slopes and the pH range over which they are buffered vary considerably. However, the titration curves of soils that have weathered under similar conditions and to approximately the same extent are quite similar.

If these curves are compared with the SiO_2-sesquioxide ratio of the soil colloids, several general tendencies are manifested. In the first place, if only those soils that are moderately or highly weathered are considered, the total exchange capacity of the colloid increases with the SiO_2-sesquioxide. This has been observed by other investigators (12 18, 19). Secondly, the buffer capacity of the colloidal material increases with this ratio. This is especially manifested by soils 707 A, B, C, and D and soil 743 when compared with soils 386, 674, 675, and 680. Finally, the strength of the soil acid, that is, its dissociation constant, also tends to increase with this ratio. These three general relationships can be drawn from the existing data with a fair degree of accuracy.

The relative strengths of these soil acids can best be expressed by multiplying the amount of acid present, that is, the exchange capacity of the colloid, by its dissociation constant. Since the dissociation constants of these acids are expressed in this study by their pK values, the exchange capacity is divided by this value. If the resulting factor is plotted against the SiO_2-sesquioxide ratio, there appears to be no one direct relationship between them. However, there appears to be three different groups of soils in which there tends to be a fairly good relationship between these two factors. If the total exchange capacity is plotted against the SiO_2-sesquioxide ratios (fig. 7) similar relationships are obtained. The same three groups of soils appear to remain intact even though the pK values of the soil acids do not fall in these same classes. The work of Mattson (14), who used five different soils, indicates that there is a direct relation between the SiO_2-sesquioxide ratio and exchange capacity. Anderson's data (1) with seven soils show that this relationship tends to be logarithmic. The results of Parker and Pate (18) with colloids from 14 soils may be interpreted in such a way as to show that this relationship is either logarithmic, or that there are two groups of colloids in which a direct relationship is obtained. All of the existing data on the relation of the absorptive properties of soil colloids to the SiO_2-sesquioxide ratio indicate that there is some relationship between these two factors, even though its exact nature cannot be accurately established.

DISCUSSION

Nature of soil acids

It is apparent from an analysis of these titration curves and the data in table 2 that the nature of soil acids varies considerably in different soils. Highly or moderately weathered soils with a high SiO_2-sesquioxide ratio tend to have a higher buffer capacity and a smaller pK value than those that have not wea-

thered so thoroughly. However, there are several outstanding exceptions to this more or less general tendency. For example, if soils 676, and 679 (fig. 6), soils 707C and 707D (fig. 5), and soil 743 (fig. 4) are compared, it is apparent that even though they have a SiO_2-sesquioxide ratio > 3 and represent soils that are fairly well weathered, there are significant differences beween their titration curves. Their buffer capacities and the strength of their acids are different. If the conditions of weathering under which these particular colloids have developed are considered, some light is thrown upon the cause of these differences. Soils 676, and 679 are the surface horizons from soils that have developed under the podzolic type of weathering. In this type of weathering, aluminum and iron leach away from the upper layers of the soil profile and tend to precipitate or settle out in the lower horizons where they encounter a less acid medium in their percolation downward. This tends toward the formation of a complex in the surface soil that will be high in silica, either as a part of the exchange complex or as free SiO_2. On the other hand, soil 743 represents the layer of accumulation from a podzolic type of soil. This horizon contains the highest percentage of colloidal material. Materials in suspension that percolate downward from the surface horizons accumulate in this zone. Therefore, its exchange complex should be expected to be different from that of the surface horizons from which materials have been removed.

Numbers 707C and 707D represent soils that have weathered under the lateritic type of weathering in which silica tends to be carried away and aluminum and iron tend to remain behind. These two soils are the least weathered horizons of this Susquehanna profile. However, several interesting relationships are manifested. Soil 707D has a SiO_2-sesquioxide ratio of 3.50. The ratio of 707C is 3.25 while that of the horizon above it is only 2.11. This shows very clearly that as the soils of the south become more highly weathered, silica is removed from the complex. There appears to be a significant change in the type of acid as the weathering processes alter these soils. From an analysis of the data of these six soils, all of which have somewhat similar SiO_2-sesquioxide ratios, but represent different types and degrees of weathering, it is apparent that there are different types of soil acids in these particular soils. This fact is further substantiated if the highly weathered soils of the south, soils 386, 674, 675, 680, 707A, and 707B, are compared with these soils previously mentioned or with the soils of the coastal plain Black Belt, soils 710, 722, and 742. These differences cannot be explained by variations in the SiO_2-sesquioxide ratio, as will be brought out later in the discussion. Pierre and Scarseth (19), in studying the relation of percentage base saturation of soils to their pH values, state that the differences in soil acids cannot be attributed to variations in this ratio. They conclude that the percentage base saturation of soils at like pH values is related to the degree of dissociation of the soil acids. The degree of dissociation of any soil acid, of course, is a function of its dissociation constant and the quantity of acid present, as previously mentioned. Its relative value can be calculated at any pH value by dividing the

measured hydrogen-ion concentration by the total amount of hydrogen present (exchangeable hydrogen).

The exchange complex of any soil and, therefore, the type of soil acid, may originate in any one or all of three ways. First, weathering processes may remove certain constituents from the original alumino-silicate minerals which are leached away leaving behind a complex that would be characteristic of the kind of weathering and independent of the parent material. Secondly, the colloidal oxides of Al, Fe, and Si that arise from a partial decomposition of the original complex may mutually flocculate and form an entirely different type of complex. Finally, there may be a precipitation of Al, Fe, and Si from solution that would give another type of complex. These three possibilities would allow the formation of different complexes dependent upon the kind and extent of weathering. This conception is also held by Gedroiz (8). Mattson (14), working with artificial zeolites, found that the nature of his precipitates depended upon the H-ion concentration. Precipitates having a high SiO_2/Al_2O_3 were obtained in the presence of Ca and Mg ions. In the absence of bases, but in a neutral or slightly acid medium the precipitate consisted largely of sesquioxides. Burgess and McGeorge (7) were able to obtain different zeolites dependent upon the reaction at which they were formed. Ross (21), in studying the mineralogy of clays, states that clay minerals may form by weathering certain elements from an old mineral; by complete replacement of a mineral; by crystallization of colloidal material; by direct precipitation from solutions; and by a reaction between older minerals and the soil solution. He further states that there is a relation between the type of clay mineral and the climatic or other physical conditions under which it is formed and that it would have different chemical and physical properties. He found, however, that there appeared to be a "dominant clay-forming mineral" of the beidellite type.

The data here presented do not confirm the conclusions of Kerr (13), who states that there is only one soil acid. However, it may be that the soils from which he extracted the "true" exchange complex were of such a nature that wide differences were not manifested. Soils from the South may compare with those from the North if the extent of weathering has not been considered.

The nature of the soil acid appears to be so closely associated with the kind and extent of weathering that it may be used, perhaps, in the classification of soils. For example, in figures 2a and 2b there appear to be two types of acids represented by four different soils. The nature of the acid in soils 675—Norfolk sandy loam—and 680—Greenville fine sandy loam—is the same, even though the Norfolk has weathered from unconsolidated sandstone and the Greenville from limestone. They are both Coastal Plain soils and have weathered to about the same extent. Soil 386—Decatur silt loam—and soil 674—Cecil clay loam—appear to have the same type of acid. The have weathered similarly and represent older soils than the Norfolk and the Greenville. The Decatur has weathered from limestone and the Cecil from silicate rocks. Likewise, in figure 6, the nature of the acid in soil 676—Colby silt loam from

Wisconsin, a soil derived from loessial material—is similar to that in soil 679—Miami silt loam, weathered from glacial till in the late Wisconsin drift. Both soils have weathered to approximately the same extent under the podzolic type of weathering. Kerr (13) has shown that the base exchange complex in these two soils is similar. Furthermore, if the soils of the coastal plain Black Belt are considered (figs. 3 and 4), the nature of the acid appears to be a criterion of the extent of weathering in these soils. These soils are derived from the same geologic deposit, namely, a heavy marine clay deposited on the Selma chalk. The Lufkin is the least weathered, has the highest SiO_2-sesquioxide ratio, and possesses the weakest acid. The Oktibbeha is the most highly weathered, has the lowest ratio, and possesses the strongest acid. This comparison holds true in comparing the surface horizons which are the most highly weathered zones in the profile.

Gedroiz (8) has suggested that the absorbed cations in the soil exchange complex may be used in a genetic classification of soils. He has divided all soils into several categories on this basis. Podzolic and lateritic soils are classified as soils containing exchangeable hydrogen in the exchange complex. The latter are characterized by a strong decomposition of the complex throughout the entire profile (true laterite) with an accumulation of Al_2O_3 and Fe_2O_3. The exchange complex of the former is decomposed only in the surface horizons. The data presented in the foregoing indicate that the nature of the soil acid as determined by an analysis of its titration curve may also serve as a basis for characterizing soils.

Soil acidity as affected by the SiO_2/R_2O_3

An analysis of the data in table 2 and figure 7 clearly shows that there is no one direct relationship between the SiO_2-sesquioxide ratio and the nature of the soil acids. This may seem contradictory to the previous work of Anderson, Mattson (15), and Parker and Pate (18). However, as previously mentioned, the results of these investigators may be interpreted in several ways dependent upon the number and kind of soils that were studied. The result in figure 7 indicate that there may be three groups of soil colloidal materials in which there tends to be a direct relationship between the SiO_2-sesquioxide ratio and the total absorptive capacity of the colloid as well as the relative strength of the soil acids. The data of Parker and Pate (18) show that there may be two such groups in their respective colloids.

A satisfactory explanation for these three "apparent groups" cannot be given because of an insufficient knowledge of the exact chemical nature of the soil exchange complex. Two possibilities, however, seem to be manifested. Following the suggestion that the soil exchange complex may form in any one or all of three ways, and that it can vary according to the kind and extent of weathering, these three "apparent groups" may be the result of different types of exchange complexes. On the other hand, different weathering processes are conducive to the accumulation of various amounts of the free oxides

of Al, Fe, and Si. SiO_2 tends to accumulate under podzolic types of weathering. Lateritic weathering is characterized by an accumulation of Al_2O_3 and Fe_2O_3. If free oxides were present the observed SiO_2-sesquioxide ratio would not represent the true ratio of the exchange complex. Mattson (15) has shown conclusively that the presence of free sesquioxides materially affects the absorptive properties of the soil. Colloidal material from a Norfolk soil that originally had a SiO_2-sesquioxide ratio of 1.63 and a total exchange capacity of 20.7 milliequivalents per 100 gm. of colloid, was found to have a ratio of 2.19 and an exchange capacity of 42.5 milliequivalents after the free sesquioxides had been removed. The results in figure 7 may be explained, therefore, on the bases that some of these colloids contained free Al_2O_3 and Fe_2O_3 and others free SiO_2 Perhaps if the true chemical composition of the exchange complex was determined, the results would show a more direct relationship.

During the process of electrodialysis the free oxides of Al and Fe moved towards the cathode and deposited on the membrane next to the cathode chamber where they encountered an alkaline reaction. This resulted in a discoloration of the membrane. Observations showed that some of these colloidal materials apparently contained no free oxides of Al and Fe because the membrane was not discolored. However, this did not indicate an absence of free SiO_2. It was interesting to note that most of the soils that apparently contained few free sesquioxides fell in the middle class in figure 7.

THE RELIABILITY OF PRESENT METHODS OF ANALYZING THE EXCHANGE COMPLEX

It is very evident from the foregoing discussion that many of the apparent discrepancies in the relation of the SiO_2-sesquioxide ratio to the absorptive properties of the exchange complex and the nature of the soil acids are associated with an incomplete understanding of the true chemical composition of the complex. Present methods for analyzing the exchange complex involve the separation of the colloidal material from the soil and the determinations of the different elements in the fraction thus obtained. Data have been obtained, thereby, that cannot be satisfactorily correlated. If the wide variations in the technique of isolating the so-called exchange complex as well as the possibilities of its containing free oxides are considered, it is apparent that satisfactory relationships between the SiO_2-sesquioxide ratio and the properties of the colloids cannot be expected.

It appears logical, therefore, to suggest that present methods be so modified that the colloidal fraction of soils as isolated by different investigators will be fairly uniform as far as its mechanical composition is concerned. In addition to this, the colloidal material should be analyzed for the free oxides of Al, Fe, and Si. This undoubtedly will require a thorough study of analytical methods. Gedroiz (8) suggests a mild KOH extraction for determining the free SiO_2 in soils. Mattson (15) determines the free sesquioxides by peptization with a hot $AlCl_3$ solution. His preliminary data show some very interesting relationships.

If the free oxides of the soil colloidal material can be accurately determined

without destruction of the exchange complex, the results obtained will undoubtedly not only give a more complete conception of the true nature of the exchange complex as developed under different conditions, but also the relation of the accumulation of free oxides to these same conditions. Until more is known concerning the effect of free oxides, satisfactory relationships between the chemical composition of the soil colloidal material and its physico-chemical properties will not be obtained.

SUMMARY

Colloidal material has been isolated from 21 different soils representing various kinds and states of weathering. The SiO_2-sesquioxide ratio, the total exchange capacity, and the nature of the soil acids have been determined on these colloids.

The following results have been obtained in this study:

The nature of soil acids varies considerably in different soils. In weathered soils their nature is solely a function of the kind and extent of weathering and is independent of the parent material. This indicates that there is more than one type of soil acid.

Colloidal material extracted from soils that are well weathered and that have a high SiO_2-sesquioxide ratio tends to be more highly buffered and exhibits stronger acidity than those colloids having a low ratio. Their total exchange capacity is higher.

The buffer capacity of the colloids appears to be primarily a function of the nature of the soil acid.

The nature of the soil acids may prove to be a valuable criterion in the classification of soils.

The exchange complex may develop by removal of certain constituents from the original alumino-silicate minerals; by mutual flocculation of the colloidal oxides of Al, Fe, and Si; and by the precipitation of Al, Fe, and Si from solution.

There is no one direct relationship between the SiO_2-sesquioxide ratio and the total exchange capacity of the colloid, or the nature of the soil acid. Three different groups appear to be manifested in which a direct relationship between these factors is indicated. It is suggested that this may be due to different types of exchange complexes, or to the presence of free oxides of Al, Fe, and Si.

It is suggested that in order to study more accurately the relation of the chemical composition of soil colloidal material to its physicochemical properties the present methods be so modified that a uniform colloidal fraction is isolated from the soil, and that the free oxides of Al, Fe, and Si are not considered in expressing the SiO_2-sesquioxide ratio.

REFERENCES

(1) ANDERSON, M. S. 1929 The influence of substituted cations on the properties of soil colloids. *Jour. Agr. Res.* 38: 521–564

(2) ANDERSON, M. S., AND MATTSON, S. 1926 Properties of colloidal soil material. U. S. Dept. Agr. Bul. 1452.

(3) BAVER, L. D. 1929 The effect of the amount and nature of exchangeable cations on the structure of a colloidal clay. Missouri Agr. Exp. Sta. Res. Bul. 129.

(4) BRADFIELD, RICHARD 1923 The nature of the chemical reactions of colloidal clay. *Colloid Sym. Monog.* 1: 369–391.

(5) BRADFIELD, RICHARD 1924 The effect of the concentration of colloidal clay upon its hydrogen-ion concentration. *Jour. Phys. Chem.* 28: 170–175.

(6) BRADFIELD, RICHARD 1928 The saturation capacity of colloidal clay soils. *Proc. First Internatl. Soil. Cong.* 4: 858–868.

(7) BURGESS, P. S., AND McGEORGE, W. T. 1927 Zeolite formation and base-exchange reaction in soils. Ariz. Agr. Exp. Sta. Tech. Bul. 15.

(8) GEDROIZ, K. K. 1929 Der adsorbierende Bodencomplex und die adsorbierten Bodenkationene als Grundlage der genetischen Bodenklassification. *Kolloidchem. Beih.* 29: 149–260.

(9) GILE, P. L. 1925 Nature of soil colloidal material. *Colloid Sym. Monog.* 3: 216–227.

(10) JOFFE, J. S., AND McLEAN, H. C. 1926 Colloidal behavior of soils and soil fertility: The soil complex capable of base exchange and soil acidity. *Soil Sci.* 21: 181–195.

(11) KELLEY, W. P., AND BROWN, S. M. 1926 Ion exchange in relation to soil acidity. *Soil Sci.* 21: 289–302.

(12) KERR, H. W. 1928 The nature of base exchange and soil acidity. *Jour. Amer. Soc. Agron.* 20: 309–335.

(13) KERR, H. W. 1928 The identification and composition of the soil alumino-silicate active in base exchange and soil acidity. *Soil Sci.* 26: 385–398.

(14) MATTSON, S. 1928 The electrokinetic and chemical behavior of the alumino-silicates. *Soil Sci.* 25: 289–312.

(15) MATTSON, S. 1928. Anionic and cationic adsorption by soil colloidal materials of varying $SiO_2/Al_2O_3 + Fe_2O_3$ Ratio. *Proc. First Internatl. Soil Cong.* 2: 199–211.

(16) ODÉN, SVEN 1927 Discussion on soil acidity. *Trans. Second Comn. Internatl. Soc. Soil Sci.* (Gröningen) B: 10–29.

(17) PAGE, H. J. 1926 The nature of soil acidity. *Trans. Second Comn. Internatl. Soc. Soil Sci.* (Gröningen) A: 232–244.

(18) PARKER, F. W., AND PATE, W. W. 1926 Base exchange in soil colloids and the availability of exchangeable calcium in different soils. *Jour. Amer. Soc. Agron.* 18: 470–482.

(19) PIERRE, W. H., AND SCARSETH, G. D. 1930 The percentage base saturation of widely different soils at definite pH values. (Unpublished paper.)

(20) ROBINSON, W. O., AND HOLMES, R. S. 1924 The chemical composition of soil colloids. *U. S. Dept. Agr. Bul.* 1311.

(21) ROSS, C. S. 1928 The mineralogy of clays. *Proc. First Internatl. Soil. Sci. Cong.* 4: 555–561.

A METHOD OF OXIDIZING AND DISSOLVING SOIL FOR THE DETERMINATION OF TOTAL AND FILTERABLE MANGANESE AND PHOSPHORUS[1]

E. M. EMMERT

Kentucky Agricultural Experiment Station

Received for publication May 16, 1930

The following method of preparing soil solutions for the determination of total and filterable manganese and phosphorus has been found to be simpler than the official methods. The colorimetric analyses used enable greater accuracy on small quantities of the elements than the older standard methods.

SOLUTION OF SMALL SAMPLES FOR TOTAL ANALYSES

Put 1 gm. of finely pulverized soil (ground to pass a 50-mesh sieve or finer) into a dry 500-cc. Kjeldahl flask. Add 2 gm. of sodium chlorate. Wash down the sides with 25 cc. of 50 per cent (by volume) H_2SO_4 and gently mix. Adjust the free flame to full height and immediately place it under the flask. This is done so that the air above the solution may be heated more rapidly than the solution and decompose the explosive chlorine peroxide as fast as it is liberated. If excessive green fumes form, care must be taken to prevent an explosion. In an open flask, however, the explosion is seldom serious. If the acid concentration is correct and the heat is applied properly, chlorine peroxide will not accumulate and no explosion will occur.

If total nitrogen is to be estimated, the flask may be connected to a distillation apparatus as described in an earlier article (3). If violent reaction begins withdraw the flame until violence ceases and then continue the heating carefully. When green fumes cease forming and the action is no longer violent, heat vigorously until the solution is clear, water has been driven off, sulfur trioxide fumes form, and sulfuric acid condenses on the sides. A green color of the solution at this point indicates manganese and proves that oxidation is complete. If the residue and solution are not free of organic matter add more chlorate in small portions at a time until the solution clears. When organic matter has been oxidized, chlorine driven off, and the solution cooled somewhat, add 75 cc. of water and shake well. If there is much residue the solution should be boiled until it is certain that all material that will dissolve has gone into solution. Make up to exactly 100 cc. The residue should be colorless and translucent if

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

all minerals have been dissolved and only siliceous material remains. If the residue settles readily, aliquots of the clear solution may be pipetted off for analysis. Filter, if necessary, and use appropriate aliquots for estimation of total manganese and phosphorus.

SOLUTION OF LARGE SAMPLES FOR TOTAL ANALYSES

If more than 1 gm. of soil is used, add 5 cc. of concentrated nitric acid and 1 to 2 gm. of chlorate for each gram of sample. Heat with a high free flame. The oxidation is much less violent than in sulfuric acid and the heating should be continuously strong, unless foaming becomes excessive. Little chlorine peroxide is formed when a high strong flame is used. Heat until the residue is dry. Cool, add about 0.1 gm. of chlorate and 5 cc. of 50 per cent (by volume) sulfuric acid for each gram of soil. Heat and expel chlorine as with smaller samples. Take up the carbon-free salts as before.

TOTAL MANGANESE

To an aliquot containing 1 to 5 mgm. of manganese add concentrated sulfuric acid to make the amount of this acid in the solution about 20 cc. Add 20 cc. of nitric acid. Make up to about 70 cc. and analyze for Mn by the official method (1) with modifications as made by Willard and Greathouse (10), as follows: Add 0.05–0.1 gm. potassium periodate and boil until a permanganate color develops. Boil one minute and add a slight excess of periodate. Keep hot 10 minutes, then make to a known volume and match with a standard. A concentration of 0.01 to 0.05 mgm. of Mn per cubic centimeter is most satisfactory for readings in a colorimeter. The standard should be made as directed by Willard and Greathouse (10), or according to the official method (1).

TOTAL PHOSPHORUS

Make an aliquot containing 0.1 to 0.5 mgm. phosphorus up to about 50 cc. and add a few drops of phenolphthalein. Add 10 per cent NaOH drop by drop until a pink color appears. Make up to 70 cc. and determine phosphorus by the method of Fiske and Subbarow (6), making a few minor changes as follows: Add 10 cc. of 2.5 per cent ammonium molybdate made up in 5 *N* sulfuric acid. Mix well and add 4 cc. of 0.25 per cent 1,2,4-aminonaphtholsulfonic acid prepared as directed by Fiske and Subbarow (6). At the same time treat a standard solution in the same manner. (Dissolve 0.1755 gm. potassium dihydrogen phosphate in 1,000 cc. of water. Treat 5 cc. of this stock solution containing 0.2 mgm. phosphorus as described in the foregoing, and dilute to 100 cc., making a solution containing 0.002 mgm. of phosphorus per cubic centimeter.) Make up the unknowns to 100 cc. and after 10 minutes compare in a colorimeter. Calculate the results to per cent of total phosphorus in the sample. The method of Deniges as used by Truog and Meyer (9) may be applied to this solution, although the foregoing method is preferred because of certain advantages (5).

RESULTS ON TOTAL MANGANESE AND PHOSPHORUS IN SOIL

Karraker[2] (table 1) used the foregoing method for destroying organic matter and dissolving manganese, and obtained more manganese from two out of three soils than Shedd did by sodium carbonate fusion (8). Karraker (7) used the chlorate method in determining manganese in his study of the effect of manganese on the quinhydrone electrode.

In order to test for manganese which might be held in the insoluble silicates, the thoroughly washed white silica residues were fused with sodium carbonate and tested for manganese by the periodate method. No traces could be detected. When the residues from three determinations were combined and fused with sodium carbonate, however, a faint trace of manganese did appear. This might, of course, have been from other sources than the insoluble silica.

TABLE 1

Comparison of Mn found by chlorate and by sodium carbonate fusion

SAMPLE	CHLORATE BY KARRAKER, PER CENT OF AIR-DRY SOIL	Na_2CO_3 FUSION BY SHEDD, PER CENT OF AIR-DRY SOIL
36552	0.077 0.075	0.050
56496	0.119 0.125	0.136
14614	0.240 0.250	0.198

TABLE 2

Total manganese and phosphorus in soils

SOIL	Mn			P		
	Determination 1	Determination 2	Error from average	Determination 1	Determination 2	Error from average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0 400	0.420	2.4	.....	.....	.....
2	0 049	0.045	4.2	.....	.....	.....
3	0.188	0.200	3.1	.....	.....	.....
4	0 300	0 312	2.0	0.222	0.222	0
5	0.180	0 170	2.9	0.091	0.091	0

Phosphorus gave almost perfect checks in all cases tried. Table 2 gives results typical of those obtained in the analyses for total manganese and phosphorus.

In order to determine whether silica might cause the phosphorus readings to be too high, as noted by Truog and Meyer (9), two 1-gm. samples of pure silica

[2] P. E. Karraker, Ky. Agri. Exp. Sta., unpublished data.

were treated as the samples were treated in the foregoing procedures, and the solution was tested for phosphorus. No blue color developed. This indicates that not enough silica was brought into solution to cause error in the test. Evidently silicic acid is dehydrated by the evaporation to dryness and sulfuric acid treatments.

For comparison, phosphorus was determined in samples of soil that had been analyzed by S. D. Averitt,[3] in the routine work of the soil survey, by the official magnesium nitrate method.

Table 3 presents five comparisons between the two methods. In every case the chlorate method gave slightly larger amounts of total phosphorus. In soils 1 and 5, containing about 0.06 per cent phosphorus, the chlorate method gave 0.007 per cent more total phosphorus than the magnesium nitrate method; in soil 4, containing abnormally large amounts of phosphorus, 0.222 per cent more phosphorus was found; and in soil 2, which is low in phosphorus, only 0.001 per cent more phosphorus was found.

TABLE 3

Comparison of total phosphorus in soils by the official magnesium nitrate method and by the chlorate method

Phosphorus as per cent of air-dry soil

SOIL NUMBER	CHLORATE METHOD			MAGNESIUM NITRATE METHOD	DIFFERENCE
	Determination 1	Determination 2	Average		
1	0.063	0.061	0.062	0.055	+0 007
2	0.033	0.033	0.033	0.032	+0.001
3	0 156	0.150	0.153	0.126	+0.027
4	1 020	1.088	1.054	0.832	+0.222
5	0.068	0.065	0 067	0.060	+0 007

FILTERABLE MANGANESE AND PHOSPHORUS

As yet there is no standard method for the determination of minerals available to plants. One investigator recommends treating the soil with 0.2 N nitric acid; another, with 0.1 N hydrochloric acid; and, a third, with 0.01 N hydrochloric acid. There are others who use ammonium chloride and citric acid as solvents. In an experiment on the effect of soil reaction on plant growth it was found that existing methods for determining the availability of minerals were not applicable, because the addition of nearly all the solvents brought all soils to approximately the same reaction and masked the effect of the varying degree of acidity.

Comber (2) states that there are three possible ways for plants to obtain soil nutrients; namely, by absorbing minerals naturally in true solution, by dissolving readily available minerals through root action, and by absorbing minute

[3] Of the chemistry department of the Agricultural Experiment Station, University of Kentucky.

colloidal particles. It is apparent that minerals in true solution or those in a fine state of colloidal division are the ones most likely to be absorbed by plants. In order to obtain comparable results as to availability of minerals in the same soil regulated to varying degrees of acidity and alkalinity by various treatments, it was decided to shake soil with water and filter the supernatant liquid through a certain grade of filter paper. The minerals present in this filtrate, including those in solution and those in a very fine state of colloidal suspension, were considered available to the plant. Prolonged shaking with a large quantity of water was avoided, since this would change the degree of acidity by dilution and bring about marked changes in the actual state of the soil. Ten minutes of shaking, allowing the material to stand a few minutes, and immediate filtering of the supernatant liquid should more nearly extract only those minerals in actual solution or in a fine colloidal state in the normal soil solution. This treatment should break up the soil films.

It is likely that the various analytical solvents and plant roots concentrate their action largely on the fine colloidal particles, and consequently a total analysis of these particles along with the minerals in actual solution should give a very close approximation to the available minerals in the soil without introducing foreign elements such as the hydrogen ion, the nitrate ion, the chloride ion, and the citrate ion.

The procedure adopted and a few of the results obtained follow.

Procedure used in determining filterable manganese and phosphorus in a uniform soil adjusted to varying hydrogen-ion concentrations

Place 100 gm. of air-dried soil in a 500-cc. Erlenmeyer or shaker bottle and add 2 cc. of distilled water for each gram of soil. Shake vigorously for 10 minutes. Allow to settle several minutes and decant upon a no. 2 genuine Whatman filter paper. Allow as little of the settled soil to go on the filter as possible. Pour back the first 10 to 20 cc. of filtrate. Evaporate a suitable aliquot of the filtrate to a small volume, taking care to keep any colloidal material well distributed by shaking before measuring. Treat the residue exactly as in the analysis of soil for total manganese and phosphorus. The amount of chlorate and the kind and amount of acid used should be regulated by the amount of organic matter to be destroyed. Usually only small amounts of chlorate and sulfuric acid are necessary.

Results on filterable manganese and phosphorus in soils

Tables 4, 5, and 6 present data which were obtained primarily to ascertain the effect of reaction on the availability of phosphorus and manganese in the soil. The tables are presented here only to verify the method used and the discussion will be limited to a comparison of duplicate treatments and samples.

Table 4 shows the amounts of filterable manganese found in plots treated with similar kinds and amounts of chemicals, but of different position in the house and to which manure was added, as indicated. There is great variation in

a few cases, but when it is considered that the samples were taken from different plots and not from the same composite sample, it seems that the variation is not excessive.

TABLE 4

Filterable manganese found in tomato soils

TREATMENT NUMBER	CHEMICALS ADDED TO PLOTS 1 AND 2		Mn		ERROR FROM AVERAGE	AVERAGE pH OBTAINED	MANURE ADDED TO PLOTS
	Kind	Tons an acre	Plot 1	Plot 2			
			p.p.m.	*p.p.m.*	*per cent*		
1	Check		0.50	0.50	0	7.04	1 and 2
2	$Ca(OH)_2$	5.5	0.25	0.28	5.7	8.52	2
3	H_3PO_4	1.0	0.25	0.56	38.3	6.42	2
4	Na_2CO_3	2.4	9.80	10.00	1.0	8.43	2
5	Na_2CO_3	0.5	2.00	2.03	0.7	7.58	2
6	HNO_3	2.4	3.10	3.10	0	5.85	1 and 2
7	Check		1.33	3.30	42.5	7.09	None
8	H_3PO_4	6.0	3.50	4.25	16.1	5.25	2
9	Check		0.49	0.49	0	6.86	None
10	Na_2CO_3	0.5	1.95	1.95	0	7.70	2
11	$CaCO_3$	18.0	0.12	0.12	0	8.00	2
12	H_2SO_4	2.5	6.22	8.44	15.1	6.15	2

TABLE 5

Filterable phosphorus found in tomato soils

TREATMENT NUMBER	CHEMICALS ADDED TO PLOTS 1 AND 2		Mn		ERROR FROM AVERAGE	AVERAGE pH OBTAINED	MANURE ADDED TO PLOTS
	Kind	Tons an acre	Plot 1	Plot 2			
			p.p.m.	*p.p.m.*	*per cent*		
1	Check		20.0	20.0	0	7.04	1 and 2
2	$Ca(OH)_2$	5.5	11.2	10.7	2.3	8.52	2
3	H_3PO_4	1.0	57.6	51.2	5.9	6.42	2
4	Na_2CO_3	2.4	98.3	89.1	4.9	8.43	2
5	Na_2CO_3	0.5	79.9	83.1	1.9	7.58	2
6	HNO_3	2.4	23.2	24.0	1.7	5.85	1 and 2
7	Check		8.2	8.6	2.4	7.09	None
8	H_3PO_4	6.0	192.0	200.0	2.0	5.25	2
9	Check		19.2	24.8	12.7	6.86	None
10	Na_2CO_3	0.5	27.2	33.6	10.5	7.70	2
11	$CaCO_3$	18.0	24.0	24.0	0	8.00	2
12	H_2SO_4	0.5	9.1	8.3	4.6	6.15	2
13	HNO_3	1.0	11.5	12.2	2.9	6.74	2
14	H_3PO_4	5.0	280.0	280.0	0	5.10	None
15	$Ca(OH)_2$	5.5	5.3	5.6	2.7	8.70	2
16	$CaCO_3$	18.0	8.0	8.3	1.8	8.00	2
17	H_2SO_4	2.5	28.3	21.7	13.2	5.61	2
18	H_2SO_4	3.0	26.4	32.0	9.5	5.20	2

Table 5 includes data on filterable phosphorus from the same plots as in table 4 and a few additional cases. The variation from the average here does not exceed 13.2 per cent and as a rule is under 5 per cent, which is good, considering that the duplicates were not run on composited samples from the same plot, but from composited samples from plots of similar treatment.

In order to check the method further, two determinations were made on a composite sample from a single plot. Table 6 shows that the amounts of filterable phosphorus in the two determinations from the same composite sample are practically identical for each treatment or hydrogen-ion concentration which existed in these soils. Table 6 also shows duplication between like treatments. For instance plots 2, 10, 16, and 18 are check plots and contain almost identical amounts of filterable phosphorus.

TABLE 6

Filterable phosphorus in soils in which lettuce was grown

Two determinations on composite samples of the same air-dry soil from each plot

PLOT	TREATMENT	TONS AN ACRE	DETERMINATION 1	DETERMINATION 2	ERROR FROM AVERAGE	AVERAGE pH OBTAINED
			p.p m.	*p.p m.*	*per cent*	
1	New soil check		11.20	.....	...	5.80
2	Old soil check		8.64	8.64	0	6.86
3	$Ca(OH)_2$	6.6	7.04	6.40	4.7	8.34
4	$CaCO_3$	17.6	7.36	7.36	0	7.84
5	$CaCO_3$	3.3	5.76	5.76	0	7.31
6	H_2SO_4	1.4	8.96	8.32	3.7	6.14
7	H_2SO_4	2.6	22.40	22.40	0	5.61
8	H_2SO_4	5.9	25.60	25.60	0	4.64
9	HNO_3	2.9	10.24	10.24	0	5.98
10	Old soil check		8.64	8.64	0	6.90
11	Na_2CO_3	3.1	27.60	27.60	0	8.76
12	Na_2CO_3	1.0	11.56	11.56	0	7.70
13	Na_2CO_3	0.5	8.96	8.64	1.8	7.31
14	H_3PO_4	1.4	48.00	48.00	0	6.23
15	H_3PO_4	3.5	115.20	115.20	0	5.75
16	Old soil check		8.64	7.68	5.9	6.85
17	H_3PO_4	8.1	264.00	.....	...	5.24
18	Old soil check		9.28	9.60	1.7	6.79
19	HNO_3	0.8	6.40	6.40	0	6.49
20	New soil + Na_2CO_3	0.33	11.20	10.88	1.4	6.84

SUMMARY

Soils are prepared for total manganese and phosphorus analyses by destroying the organic matter and bringing the minerals into solution by heating with sulfuric acid and sodium chlorate.

Findings for manganese and phosphorus are presented.

The procedure is simpler than the usual methods and the danger of loss of the elements may be less.

A method for determining relative availability of manganese and phosphorus without introducing foreign elements is proposed. The soil is shaken for 10 minutes with water allowed to stand a few minutes, and the supernatant liquid filtered through a genuine Whatman no. 2 filter paper. The filtrate, which may include colloidal particles as well as substances in solution, is assumed to contain the approximate amount of manganese and phosphorus compounds that are available as plant-food. Manganese and phosphorus are determinated in this liquid in nearly the same way as described for total analysis.

REFERENCES

(1) Changes in the Official and Tentative Methods of Analysis Made at the 44th Annual Convention, Oct. 29–31, 1928. 1929 *Jour. Assoc. Off. Agr. Chem.* 12: 36.

(2) Comber, N. M. 1927. Scientific Study of the Soil, ed. 1, p. 127. Edward Arnold and Company, London.

(3) Emmert, E. M. 1929. The chlorate method for the determination of nitrate nitrogen, total nitrogen, and other elements in soils and plant tissues. *Jour. Assoc. Off. Agr. Chem.* 12: 241–247.

(4) Emmert, E. M. 1930 Procedure for determining total nitrogen by conversion to nitric acid. *Jour. Assoc. Off. Agr. Chem.* 13: 146–147.

(5) Emmert, E. M. 1930 A method for the determination of phosphate phosphorus in green plant tissues. *Plant Physiol.* 5: 413–417

(6) Fiske, C. H., and Subbarow, Y. 1925 The colorimetric determination of phosphorus. *Jour. Biol. Chem.* 66: 388.

(7) Karraker, P. E. 1930 The inaccuracy of the quinhydrone electrode in many Kentucky soils. *Jour. Amer. Soc. Agron.* 22: 171–180.

(8) Shedd, O. M. 1914 The occurrence of manganese in Kentucky soils and its possible significance. *Jour. Indus. and Engin. Chem.* 6: 662.

(9) Truog, E., and Meyer, A. H. 1929 Improvements in the Deniges colorimetric method for phosphorus and arsenic. *Indus. and Engin. Chem.* (Analyt. ed.) 1: 136.

(10) Willard, H. H., and Greathouse, L. H.: 1917 Colorimetric determination of manganese by oxidation with periodate. *Jour. Amer. Chem. Soc.* 39: 2366.

HYDROGEN-ION CONCENTRATION, ALUMINUM CONCENTRATION IN THE SOIL SOLUTION, AND PERCENTAGE BASE SATURATION AS FACTORS AFFECTING PLANT GROWTH ON ACID SOILS[1]

W. H. PIERRE

Alabama Agricultural Experiment Station

Received for publication May 5, 1930

Much investigation of soil acidity in its relation to plant growth has resulted in the proposal of a number of theories for explaining the data obtained. Although it is realized that soil acidity may affect plant growth in a variety of ways, as has been pointed out by Truog (44), the question as to which factors of acidity are directly concerned and are of primary importance remains to be determined. Robinson (37) recently expressed this viewpoint as follows: "In so-called acid soils we have a combination of acid reaction, active aluminum, low calcium content, and other factors all more or less correlated among themselves. It is important to know which of these factors is the primary factor of which the others are only secondary consequences."

Since the introduction of the hydrogen electrode into soil investigations, much has been claimed for the value of determinations of hydrogen-ion concentration in explaining the relationship between soil acidity and plant growth (3, 24, 39, 40, 46). Various lines of evidence suggest, however, that the hydrogen-ion concentration of soils cannot be considered a direct or primary factor in causing poor plant growth on acid soils. In the first place, conflicting results have been obtained by various investigators (7, 26, 36, 42) regarding the optimum reaction for the growth of the same crops. Secondly, several investigators (12, 13) have reported little correlation between hydrogen-ion concentration of soils and the response of crops to liming.

Both Duley (12) and Fleetwood (13) conclude from their works that the amount of available soil calcium as determined by extraction with carbonated water, is a better indication of the response of plants to liming than is the total acidity of the soil or its hydrogen-ion concentration.

On the other hand, much evidence has been submitted in favor of the toxic aluminum theory of soil acidity by a number of American investigators (4, 8, 10, 22). Without going into any detail regarding the investigations upon which this theory is based, the recent criticism of the theory by Line (17) raises some question as to its validity.

[1] Published with the approval of the director of the Alabama Agricultural Experiment Station. Part of the work connected with this paper has been done since the writer has been with the West Virginia Agricultural Experiment Station.

Moreover, the recent development of the base exchange conception of soil acidity suggests that plant growth on acid soils may be more closely related to the condition of the soil with respect to base saturation than it is to hydrogen-ion concentration or aluminum toxicity.

This investigation was started with the following objectives:

To compare different soils with respect to the hydrogen-ion concentration at which plant growth is definitely reduced ("critical hydrogen-ion concentration").

To determine the concentration of aluminum and other elements in the displaced soil solution at various degrees of acidity and to study their effect on plant growth.

To study the relationship between plant growth on acid soils and the ratio of potassium to calcium in the displaced soil solution.

To study the influence of the percentage base saturation of acid soils on plant growth.

EXPERIMENTAL METHODS AND PROCEDURE

Thirteen large samples of soils were obtained, seven from Alabama and the remainder from four other states.[2] They were selected with the purpose of having soils formed under different climatic conditions and which were of different degrees of weathering. A description of the soils is given in table 1. Each soil was thoroughly mixed and passed through a one-fourth inch screen upon being taken to the greenhouse. Seven kilograms of dry soil was then placed in each of ten 2-gallon glazed earthenware pots. This provided two pots for each of the five different soil treatments to be given.

Treatment of soils

The different treatments consisted of bringing each soil to five different degrees of reaction. This was done by additions of lime or of a combination of nitric and sulfuric acids. In all cases but one the soils had original reactions between pH 5.20 and 6.00. Three of the treatments consisted of additions of a combination of equal amounts of normal nitric and sulfuric acids to bring the soils to pH values of 4.80 to 5.10. These acids are believed to simulate the action of ammonium sulfate on soils. The other treatment consisted of liming each soil according to its respective content of exchangeable hydrogen, determined according to the barium acetate method (27). The other set of pots received no lime nor acid treatments. In the case of soil 671, all were lime treatments, since the soil had an original pH value of 4.40. The amount of acid required to bring the soils to the pH values desired was determined by the buffer method previously described (31). The acid was added by dumping the soil from a pot into a large pan and by adding the acid in a fine spray while mixing the soil in the pan. The lime was added in the form of precipitated calcium carbonate. After the soils were put back into the pots, rain water was added to bring them to approximately optimum moisture. They were kept

[2] The writer wishes to express his appreciation to the following men for furnishing soil samples from their respective states: Prof. W. E. Ayres, Mississippi; Dr. F. C. Bauer, Illinois; Dr. T. S. Buie, South Carolina; Prof. A. R. Whitson, Wisconsin.

at about this moisture content for six months by further additions of rain water when necessary. At the end of two and four months the soil in each pot was mixed thoroughly and a sample taken for determination of hydrogen-ion concentration. At the end of six months the soils were leached with rain water,

TABLE 1

Description of soils used in this investigation

SOIL NUMBER	SOIL TYPE	GEOLOGIC CLASSIFICATION OF SOILS	H-ION CONCENTRATION	ORGANIC MATTER (ORGANIC C × 1.724)	TOTAL EXCHANGE CAPACITY	$\frac{\text{mols } SiO_2}{\text{mols } R_2O_3}$ OF COLLOIDS
			pH	*per cent*	*mgm. equiv.*	
670	Grundy silt loam (Ill.)	Derived from Peorian loess under conditions of poor drainage	5.20	6 07	29.37	2.99
671	Cory silt loam (Ill.)	Derived from shallow deposits of Sanganon and Peorian loess on top of Illionian glacial drift	4.58	1.91	8.88	3.20
672	Cecil sandy loam (S. C.)	Residual. Piedmont Plateau	5 88	3.48	7.29	1.78
673	Delta light silt loam (Miss.)	Alluvial, from Miss. River	6.60	1.48	9.47	2.66
674	Cecil clay loam (Ala.)	Residual. Piedmont Plateau	6.15	1.57	5.57	1.57
675	Norfolk sandy loam (Ala.)	Marine. Coastal Plains	5.60	0.97	2.35	1.92
676	Colby silt loam (Wis.)	Glacial and loessial	5.63	3.64	14.54	3.18
678	Cecil clay (Ala.)	Residual. Piedmont Plateau	5.43	1.60	4.35	1.48
679	Miami silt loam (Wis.)	Glacial till	5.85	3.36	10 68	3.97
680	Greenville fine sandy loam (Ala.)	Marine. Coastal Plains	5.40	2.62	8.26	1.62
683	Susquehanna fine sandy loam (Ala.)	Marine. Coastal Plains	5.40	2.47	5.12	2 01
684	Norfolk sandy loam (Ala.)	Marine. Coastal Plains	5.35	1.57	3.22	—
685	Greenville sandy loam (Ala.)	Marine. Coastal Plains	5.48	1.26	2.43	—

to remove the soluble salts which accumulated through the reaction of the acid with the soil, and thus to simulate field conditions as closely as possible. Seven to twelve liters of water was added to each pot for the different soils, the amount of water added to each pot of the same soil being the same. This leaching was

sufficient to remove practically all the nitrates that had been added to many of the pots in the form of nitric acid. After the soil in each pot had dried partially, it was again dumped into a large pan, thoroughly mixed, and a sample taken for determination of hydrogen-ion concentration. The soils were then fertilized, as described elsewhere in this paper.

Soils 683, 684, and 685 were added to the study later; and sorghum, the second crop grown on the other soils, was the first crop grown on these soils.

Crops grown

The crops grown, in the order of their succession, were corn, sorghum, and barley. Barley and sorghum are known to be sensitive to soil acidity, whereas corn is usually considered fairly acid resistant. The corn was planted May 26, 1928, and harvested July 4. Sorghum was planted August 1, 1928, and harvested when the crop was mature, October 4. Barley was planted November 24, 1928, and harvested in May, 1929. With corn and sorghum three stalks were grown in each pot, whereas with barley five plants were grown in each pot.

Fertilizer treatment of the soils

It seemed best in this study to fertilize all the soils with phosphorus, nitrogen, and potash so that they might be removed as limiting factors and also that any indirect effects of soil reaction on the availability of these elements might be decreased. Before the planting of each crop, the soils were thoroughly mixed with the fertilizer. The phosphate fertilizer was applied for each crop at the rate of 160 pounds of P_2O_5 for each acre of 2,000,000 pounds of soil. For the corn crop this was in the form of superphosphate; for the other two crops monocalcium phosphate was used as the source of phosphorus. The potassium fertilizer was used in the form of potassium nitrate at the rate of 100 pounds an acre for corn and barley, and 135 pounds an acre for sorghum.

In order to prevent the accumulation of large amounts of soluble salts, most of the nitrogen fertilizer was applied at various times during the growing period rather than all at planting time. The amounts and kinds of nitrogen fertilizers applied to each crop are given in table 2. In order to maintain the soils at the desired reactions, a combination of nitrogenous fertilizers that should have no effect on the soil reaction was used (32). This was a combination of physiologically basic nitrogen fertilizer with an acidic fertilizer, ammonium nitrate, in the proportion of two parts of nitrogen in the former and three parts in the latter. Since after the calculated acid treatment, a few of the soils did not have pH values as low as was desired, these received a greater proportion of their nitrogen in the form of acidic sources, as is shown in table 2. The soils upon which plants showed very poor growth or decided acid injury did not receive the additional nitrogen fertilizers applied during the growing period. For the sorghum crop these were pots 3 and 4 of series 672 and 678, pots 3 to 6 inclusive

of soil 684, and pots 3 to 8 inclusive of soil 675. For barley the following pots received only one-third as much nitrogen after planting as is indicated in table 2: pots 3 and 4 of soils 672, 675, 678, and 680; and pots 5 and 6 of soil 675. This was thought necessary in order to prevent the accumulation of excess soluble salts which would be likely to accentuate the injury to the plants.

TABLE 2

The amounts and forms of nitrogen applied for the various crops

(In pounds for each 2,000,000 pounds of soil)

SOILS NUMBER	AMOUNTS OF NITROGEN APPLIED IN DIFFERENT FORMS						
	At planting		During growing period				Total
	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	$NaNO_3$	NH_4NO_3	$(NH_4)_2SO_4$	
For corn							
All but Nos. 670, 673, and 679	13 8		36.2		50		100
Nos. 670, 673, and 679	13 8		11 2		75	40	140
For sorghum							
All except Nos. 670, 673, 676, and 679	18 7	28 0		33 3	50.0		130
Nos. 670, 673, 676, and 679	18.7	71 3				40.0	130
For barley							
All soils	13.8	20 7		36.0	54 0		125

Soil studies

Soil studies consisted of the following: determination of the hydrogen-ion concentration of the soil from each pot taken before and after harvesting each crop; determination of aluminum, phosphorus, calcium, potassium, and manganese in the displaced soil solution from each pot obtained after the growth of corn and before fertilizing for sorghum; and determinations of the percentage base saturation of the soils sampled after the growth of corn and before displacing the soil solution. The soil solutions were obtained by the displacement method (27). For this purpose about 2,000 gm. of soil was used from each pot. After displacement, the soils were put back into their respective pots, allowed to dry partially, and then mixed thoroughly with the soil left in the pots.

Chemical methods

The hydrogen-ion concentration of the soils was determined by the "dialysis colorimetric method" (34). When the necessary precautions were taken, this method was shown to give accurate results (33).

The percentage base saturation values of the soils were based on the determinations of exchangeable hydrogen according to Parker's (28) barium acetate method and on the determinations of total exchange capacity according to the $Ba(C_2H_3O_2)_2$-NH_4Cl method (28).

The phosphate content of the soil solution was determined by the blue colorimetric method (29); potassium by the cobalt nitrite colorimetric method as modified by Tidmore (41); and calcium and manganese were determined by the standard methods.

The aluminum content of the soil solution was determined by the "aluminon" method recently proposed by Yoe and Hill (47). After a thorough examination of the method and the adoption of a few slight changes, it was found to be satisfactory. The changes included: the addition of 2 cc. of a 20 per cent ammonium chloride solution before precipitating the iron and aluminum in order to make the separation more complete, the use of a series of standards in which the color is developed at the same time as in the unknown solution, the taking of readings between 20 and 30 minutes after developing the color. It was found that if a longer time than this is allowed, the color in the unknown solutions which have been subjected to the procedure for removal of the iron, fades more quickly than does that of the standards. The recovery of aluminum when added in concentrations of 0.5, 1.0, and 2.0 p.p.m. averaged about 97 per cent. Iron, if not precipitated, was found to interfere even when present in concentrations of only one-fourth that of aluminum. However, by removal of the iron, according to Yoe and Hill, good recoveries of aluminum were obtained even when iron was originally present in equal concentrations. Although most of the soil solutions analyzed apparently contained very little iron, as indicated by the color of the precipitate, the procedure for removal of iron was employed in all cases. A comparison of the "aluminon" method with the gravimetric method of Lundell and Knowles (19) gave the following results on an acid soil extract: "Aluminon method," 3.2 p.p.m.; gravimetric method, 2.9 p.p.m.

EXPERIMENTAL DATA

In the presentation of these data a brief description of the characteristic plant injury noted from soil acidity is first given. The data for each of the various factors of soil acidity in its relation to plant growth are then presented in the following order: hydrogen-ion concentration, aluminum toxicity, potassium-calcium ratio in the soil solution, and percentage base saturation. As far as possible, the various factors will be considered in relation to one another. For this purpose a summary table (table 6) was prepared, to which references are made throughout the paper.

In this study the yields of sorghum are no doubt the most valuable in indicating the effect of the various factors of soil acidity on plant growth, for more soils can be compared, and the depressions in yields are much more pronounced than with corn. The yields of barley were also markedly depressed by acidity, but since the determination upon which the data in table 6 are based were made

on the soil before the sorghum crop was planted, some of these values may not hold very strictly for the soil at the time the barley crop was growing. Moreover, the barley crop did not make as vigorous and normal growth as did the sorghum. These facts should be kept in mind in the following discussion.

TABLE 3

Notes on the early growth of sorghum as affected by soil acidity

SOIL NUMBER	INJURY TO SEEDLINGS 1 WEEK OLD (BY POTS)	RELATIVE GROWTH, BASED ON 100, FOR PLANTS IN POTS 9 AND 10 (PLANTS 23 DAYS OLD)				NOTES ON APPEARANCE AND EXTENT OF INJURY WHEN PLANTS WERE 23 DAYS OLD
		Pots 1–2	Pots 3–4	Pots 5–6	Pots 7–8	
		*	*	*		
671	1, 2, 3, 4	15	25	30		Plants in pots 1 and 2 are chlorotic but less so than some of the plants in soils 672, 674, and 675 A slight blotching with reddish brown spots on leaves in pots 3 and 4
672	3, 4	100	20	40	100	Plants in pots 3 and 4 are very chlorotic. Yellow stripes along veins of leaves, with some reddish brown mottling. Leaves of these plants have wilted appearance. Plants in pots 5 and 6 less injured than those in pots 3 and 4
674	3, 4	100	40	90	100	Leaves of plants in pots 3 and 4 are chlorotic, striped, and mottled as are those described from soil 672. Some mottling in plants of pots 5 and 6
675	3, 4, 5, 6	100	5	5	20	Plants in pots 3 to 6 are very chlorotic. Leaves are turning brown at tips, otherwise similar to plants in soil 672 Plants in pots 7 and 8 slightly chlorotic
678	3, 4	100	20	100	100	Plants in pots 3 and 4 have pale green or mottled appearance, but are not wilted in appearance or mottled as are those of plants in soil 672, 674, and 675
680	3, 4	100	90	100	100	Plants in pots 3 and 4 are slightly chlorotic, striped, and blotched in appearance along the edges of the leaves
684		100	50	60	100	Plants in pots 3 to 6, although chlorotic, do not show the mottled appearance as do those in soils 672, etc.
685		100	30	100	100	Plants in pots 3 and 4 are beginning to show distinct yellowing and blotching

Plants in pots 3, 4, 5, and 6 of soil 679 showed injury when 1 week old, but this injury was overcome later. The plants grown in all the other soils not included in this table did not show injury and were apparently making good growth (see also tables 4, 5, and 6).

* Based on pots 7 and 8 as 100.

Characteristics of the plant injury from soil acidity

Plant injury on acid soils is indicated not only by the total yields obtained but also by the appearance of the plants at early stages of growth. In these experiments, the plants grown on some of the acid soils first showed injury from acidity not in growth, but in the appearance of the leaves. With corn the characteristic injury to the seedlings was noted when they were less than 1 week old. The plants were just as tall as those grown on the less acid soils, but there was present a yellowing of the leaves in stripes between the veins. In extreme cases of injury a large part of the leaf had this light yellow color. As the plants grew older, the injury to the leaves disappeared and the new leaves were normal except in extreme cases of injury. In some cases the striation persisted for several weeks without apparent injury to growth.

Notes were also made of the appearance of the sorghum plants and of their relative growth during the early growing period. A brief summary of these is given in table 3. As in the case of corn, the seedlings grown on some of the most acid soils had the characteristic loss of chlorophyll in stripes between the veins when they were only 5 to 6 days old. The plants showing this abnormal condition are indicated in table 3. During the second week of growth slight differences in the size of the plants began to be apparent. The relative growth of the plants grown on each soil was estimated when they were 23 days old. It will be noted that the relative growth of the plants showing acidity injury, as compared with the plants grown on the limed soils, was in some cases much more than is shown in the final yields. This indicates, as was also observed for the corn and barley crops, that injury to growth in the early stages of growth is sometimes largely overcome in the later stages. Yields at maturity, then, may not always truly represent the extent of the injury. In the field where conditions for growth may be less favorable this early injury would likely have a greater influence on the final yields than under greenhouse conditions.

The acidity injury to barley was also evident in the appearance of the leaves when the plants were very small. The injury was first seen as small yellowish to brown spots and stripes on the leaves especially near their tips. Then the tips of the leaves dried up entirely. As with the other crops, the injury tended to disappear as the plants got older. Whether or not the cause of this early injury is the same as that which caused a reduction in total growth is not known. It is probable that as the plants get older they establish a better root system and are thus able to withstand greater degrees of acidity.

The hydrogen-ion concentration of soils as a factor in plant growth

Various lines of evidence have already been mentioned which suggest that the hydrogen-ion concentration of soils cannot be considered a direct or primary factor in causing poor plant growth on acid soils. In some preliminary studies by the writer in relation to the development of soil acidity by the continued use of acidic nitrogen fertilizers, it was found that one of the soils studied pro-

duced just as good yields of sorghum at pH 4.60 as at any higher pH value; whereas with another soil the sorghum plants showed definite injury and marked reduction in growth as the hydrogen-ion concentration was reduced

TABLE 4

The relation between H-ion concentration of soils and plant growth

SOIL	POT NUMBER	pH VALUE OF SOILS (BEFORE CORN)	YIELDS OF CORN	pH VALUE OF SOIL (BEFORE SORGHUM)	YIELDS OF SORGHUM	pH VALUE of SOILS (BEFORE BARLEY)	YIELDS OF BARLEY	pH VALUE OF SOILS (AFTER BARLEY)
			gm.		*gm.*		*gm.*	
670 Grundy silt loam	1–2	5.20	18.7	5.20	52.8	5.00	20.8	5.03
	3–4	4.83	25.9	4.75	62.8	4.60	20.1	4.55
	5–6	4.98	23.9	4.85	62.0	4.73	17.6	4.75
	7–8	5.08	21.3	4.98	61.0	4.83	20.1	4.78
	9–10	6.60	17.6	6.40	55.6	6.03	21.5	5.98
671 Cory silt loam	1–2	4.58	11.1	4.43	10.0	4.43	12.6	4.43
	3–4	4.63	9.6	4.60	25.6	4.60	17.0	4.55
	5–6	4.90	17.5	4.85	31.6	4.80	18.3	4.78
	7–8	6.18	19.4	6.00	46.6	6.00	21.6	5.88
672 Cecil sandy loam	1–2	5.88	17.9	5.60	29.2	5.78	17.7	5.75
	3–4	4.80	14.3	4.80	5.2	4.80	6.5	4.78
	5–6	5.13	16.9	4.93	23.9	5.08	13.4	5.03
	7–8	5.25	16.8	5.08	32.4	5.30	25.5	5.10
	9–10	6.63	15.7	6.50	30.6	6.35	18.8	6.15
673 Delta light silt loam	1–2	6.60	25.2	5.80	50.9	5.65	25.2	5.78
	3–4	5.00	30.4	4.60	49.3	4.60	22.5	4.80
	5–6	5.23	33.1	4.78	51.7	4.75	20.4	4.88
	7–8	5.50	35.0	4.85	49.7	4.83	20.7	4.98
	9–10	6.83	27.0	6.55	48.7	5.95	19.3	6.00
674 Cecil clay loam	1–2	6.15	13.5	5.80	27.9	5.83	17.8	5.83
	3–4	4.73	11.6	4.75	19.2	4.80	7.1	4.83
	5–6	4.83	14.3	4.85	26.3	4.95	10.0	4.93
	7–8	4.98	15.7	4.95	32.1	5.05	16.8	5.05
	9–10	6.80	13.3	6.78	23.1	6.60	19.0	6.63
675 Norfolk sandy loam	1–2	5.60	12.9	5.40	20.6	5.60	9.1	5.63
	3–4	4.73	5.1	4.75	0.4	4.75	1.3	5.05
	5–6	4.90	8.6	4.93	0.7	4.90	1.1	5.10
	7–8	5.05	11.4	5.03	6:8	5.18	11.9	5.15
	9–10	6.58	13.5	6.43	26.9	6.20	14.2	6.10
676 Colby silt loam	1–2	5.63	20.0	5.53	50.5	5.43	20.6	5.45
	3–4	4.70	15.5	4.80	41.3	4.60	9.8	4.63
	5–6	5.00	17.6	4.95	39.1	4.80	15.8	4.78
	7–8	5.15	14.2	5.05	47.4	4.95	20.2	4.95
	9–10	6.68	20.3	6.58	52.3	6.28	21.4	6.28

TABLE 4—*Concluded*

SOIL	POT NUMBER	pH VALUE OF SOILS (BEFORE CORN)	YIELDS OF CORN	pH VALUE OF SOIL (BEFORE SORGHUM)	YIELDS OF SORGHUM	pH VALUE OF SOILS (BEFORE BARLEY)	YIELDS OF BARLEY	pH VALUE OF SOILS (AFTER BARLEY)
			gm.		gm.		gm.	
678 Cecil clay	1–2	5.43	11.0	5.38	23.3	5.38	18.9	5.38
	3–4	4 58	3.4	4.45	0.7	4.43	3.4	4.50
	5–6	4.75	9.7	4.80	19.8	4.63	15.9	4.58
	7–8	4.88	11.3	4.93	22.8	4.98	20.4	4.80
	9–10	6.60	9.3	6.50	23.6	6.35	19.7	6.25
679 Miami silt loam	1–2	5.85	29.0	5.40	58.7	5.50	21.7	5.53
	3–4	5.15	29.6	4.93	50.3	4.63	16.3	4.58
	5–6	5.40	30.7	5.03	51.5	4.80	19.6	4.73
	7–8	5.45	26.7	5.03	57.5	4.93	22.5	4.85
	9–10	6.65	29.1	6.45	57.6	6.20	24.2	6.08
680 Greenville fine sandy loam	1–2	5.40	17.3	5.30	30.6	5.40	19.5	5.38
	3–4	4.85	16.2	4.75	26.8	4.65	3.1	4.73
	5–6	4.98	18.7	4.80	27.5	4.78	18.8	4.78
	7–8	5.20	18.1	4.95	27.6	4.88	19.6	5.00
	9–10	6.65	17.9	6.40	34.8	6.35	17.9	6.10
683 Susquehanna fine sandy loam	1–2			5.40	34.6	5.35	19.2	5.40
	3–4			4.80	28.4	4.65	13.5	4.68
	5–6			4.85	28.5	4.88	17.1	4.73
	7–8			5.00	29.9	5.18	21.3	4.90
	9–10			6.20	35.2	6.00	21.3	5.95
684 Norfolk sandy loam	1–2			5.35	17.1	5.80	15.7	5.20
	3–4			4.78	2.1	4.60	13.2	4.70
	5–6			4.83	7.2	4.78	13.3	5.03
	7–8			4.95	17.6	5.40	16.3	5.33
	9–10			6.40	19.1	6.40	15.1	6.40
685 Greenville sandy loam	1–2			5.48	29.3	5.80	15.1	5.68
	3–4			4.95	13.6	4.95	13.5	4.83
	5–6			5.00	22.1	5.20	15.6	5.10
	7–8			5.15	24.0	5.40	16.5	5.10
	9–10			6.48	23.7	6.40	11.6	6.40

to pH 5.0 or below. The data presented in this investigation substantiate these earlier results.

In table 4 are given the yields of corn, sorghum, and barley grown on the different soils each maintained at five degrees of reaction The pH values of the soils before and after the growth of each crop are also given. It may first be noted that corn grew fairly well on many of the soils at low pH values, whereas sorghum and barley showed much greater injury from acidity. Even in the case of corn, however, some differences were obtained in the hydrogen-ion

concentration at which plant growth was materially reduced on the different soils. For example, soil 675, at pH 4.75 to 4.90 produced very poor yields of corn, whereas most of the other soils produced good yields at these pH values; in fact, soils 670, 673, and 679 gave the greatest yields at the most acid reactions.

If the yields of sorghum are considered, it will be noted that the critical hydrogen-ion concentration varies considerably with the different soils. For example, no depression in yields was obtained with soil 670 even at reactions. as low as pH 4.60 to pH 4.75, and practically no depression in yields on soil 673 when as low as pH 4.60; whereas on soil 672 the yield at pH 4.80 was less than 20 per cent of that obtained at higher pH values, and on soil 675 even at pH values slightly above 5.0 the yield was only about 25 per cent of that obtained at pH 6.58. The lack of correlation between hydrogen-ion concentration and plant injury is further shown in table 6. At the uniform pH value of 4.80, it will be noted that the average relative yields of the three crops ranged from 19 to 98 per cent of that obtained from the best culture of the respective series. Similar results are seen to obtain with the growth of barley.

It is thus evident that the hydrogen-ion concentration of soils cannot be considered the controlling factor in determining the growth of plants on acid soils. These data also show the false conclusions that may be drawn when working on a problem of this nature with only a limited number of soils, especially if these soils are of similar origin. If in this study only certain of these soils had been used, the results might have indicated a close correlation between the hydrogen-ion concentration and plant growth. It seems probable that one of the reasons for the good correlation obtained by various investigators between hydrogen-ion concentration and plant growth is that they worked with only few soils or with soils of very similar origin. Under such conditions it is probable that the hydrogen-ion concentration is correlated with or influences other factors which may directly affect growth, (6, 14, 18, 23, 44).

The relation of the concentration of aluminum and other elements in the soil solution to plant growth on acid soils

Many of the investigations on the toxic effect of aluminum on plant growth have been conducted in culture solutions. The limited amount of work carried out with soils has been with relatively few soils, and under such conditions that it has been difficult to isolate the aluminum factor from other factors generally associated with it. If aluminum is the main factor in determining plant injury on acid soils, one would expect to find a relationship between the amounts of aluminum in the displaced soil solution and plant growth. In the fourth column of table 5 are given the data for the concentrations of aluminum in the displaced soil solutions of the various soils at different pH values. It will be noted that the concentrations of aluminum in the soil solutions are in most cases very small even at low pH values. This is in accord with some results by Magistad (23). As was to be expected it was found that the greater the hydrogen-ion concentration of a particular soil, the greater is the concentration of

aluminum in its displaced soil solution. When the different soils are compared, however, it will be observed that the aluminum concentration in their displaced

TABLE 5

The concentration of aluminum and other elements in the soil solution and the percentage base saturation of soils as related to the yield of sorghum

SOIL	POT NUMBER	H-ION CONCENTRATION	CONCENTRATION IN THE DISPLACED SOIL SOLUTION				K/Ca IN SOIL SOLUTION	PERCENTAGE BASE SATURATION	YIELD OF SORGHUM
			Al	PO_4	Ca	K			
		pH	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*			*gm.*
670 Grundy silt loam	1–2	5.20	T	0.68	522	10.4	0.020	63.2	52.8
	3–4	4.75	T	0.60	516	14.6	0.028	56.7	62.8
	5–6	4.85	T	0.56	510	12.8	0.025	58.6	62.0
	7–8	4.98	T	0.72	688	12.8	0.019	60.5	61.0
	9–10	6.40	T	0.52	570	9.1	0.016	89.3	55.6
671 Cory silt loam	1–2	4.43	5.7	0.27	308	26.4	0.086	14.3	10.0
	3–4	4.60	2.7	0.12	616	27.4	0.044	29.1	25.6
	5–6	4.85	0.8	0.17	538	19.6	0.036	32.3	31.6
	7–8	6.00	T	0.24	808	14.2	0.017	73.6	46.6
672 Cecil sandy loam	1–2	5.60	T	0.24	538	29.6	0.055	39.1	29.2
	3–4	4.80	1.1	0.14	444	63.8	0.144	10.0	5.2
	5–6	4.93	0.5	0.17	538	34.2	0.063	18.2	23.9
	7–8	5.08	T	0.20	544	39.2	0.072	22.7	32.4
	9–10	6.50	T	0.24	548	27.4	0.059	69.1	30.6
673 Delta light silt loam	1–2	5.80	T	9.00	782	34.2	0.043	90.4	50.9
	3–4	4.60	1.0	6.80	678	41.0	0.060	71.3	49.3
	5–6	4.78	0.4	8.80	646	38.8	0.060	76.6	51.7
	7–8	4.85	T	9.00	774	39.9	0.051	77.7	49.7
	9–10	6.55	T	8.50	964	32.9	0.034	93.1	48.8
674 Cecil clay loam	1–2	5.80	T	0.07	364	25.1	0.069	51.5	27.9
	3–4	4.75	0.4	0.05	316	38.8	0.122	2.1	19.2
	5–6	4.85	T	0.07	290	31.3	0.108	10.3	26.3
	7–8	4.95	T	0.13	306	24.0	0.078	20.6	32.1
	9–10	6.78	T	0.06	470	21.7	0.046	81.4	23.1
675 Norfolk sandy loam	1–2	5.40	T	1.36	880	68.4	0.078	29.0	20.6
	3–4	4.75	3.7	0.88	706	257.0	0.364	7.1	0.4
	5–6	4.93	1.6	1.02	544	149.4	0.274	12.6	0.7
	7–8	5.03	0.8	1.18	799	86.7	0.108	29.0	6.8
	9–10	6.43	T	1.38	862	68.4	0.079	55.2	26.9
676 Colby silt loam	1–2	5.53	T	0.17	676	13.5	0.020	54.4	50.5
	3–4	4.80	1.1	0.16	424	41.6	0.098	31.5	41.3
	5–6	4.95	0.4	0.16	538	17.3	0.032	35.2	39.1
	7–8	5.05	T	0.18	564	14.4	0.025	39.0	47.4
	9–10	6.58	T	0.15	904	10.9	0.012	78.9	52.3

TABLE 5—*Concluded*

SOIL	POT NUMBER	H-ION CONCENTRATION	CONCENTRATION IN THE DISPLACED SOIL SOLUTION				K/Ca IN SOIL SOLUTION	PERCENTAGE BASE SATURATION	YIELD OF SORGHUM
			Al	PO_4	Ca	K			
		pH	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*			*gm.*
679 Miami silt loam	1–2	5.40	T	1.60	640	22.8	0.035	60.7	58.7
	3–4	4.93	T	1.10	420	51.3	0.124	47.4	50.3
	5–6	5.03	T	1.40	790	27.4	0.034	49.4	51.5
	7–8	5.03	T	1.64	883	21.7	0.024	52.0	57.5
	9–10	6.45	T	1.16	892	16.0	0.018	81.1	57.6
680 Greenville fine sandy loam	1–2	5.30	T	0.31	376	22.8	0.060	26.8	30.6
	3–4	4.75	0.4	0.21	254	29.6	0.116	16.8	26.8
	5–6	4.80	0.5	0.25	388	27.4	0.071	18.2	27.4
	7–8	4.95	T	0.27	388	26.3	0.068	20.4	27.6
	9–10	6.40	T	0.29	568	21.7	0.038	68.5	34.8
683 Susquehnana fine sand	1–2	5.40	T	0.98	280	68.4	0.244	45.7	34.6
	3–4	4.80	2.2	1.00	344	88.4	0.257	28.4	28.4
	5–6	4.85	1.4	1.04	374	85.5	0.228	36.5	28.5
	7–8	5.00	0.9	0.90	380	71.3	0.187	41.5	29.9
	9–10	6.20	T	0.91	470	57.0	0.121	68.6	35.2
684 Norfolk sandy loam	1–2	5.35	T	0.15	368	71.3	0.193	34.3	17.1
	3–4	4.78	1.4	0.10	230	114.0	0.495	17.7	2.1
	5–6	4.83	1.0	0.10	268	88.4	0.329	22.3	7.2
	7–8	4.95	0.8	0.15	372	65.6	0.176	31.0	17.6
	9–10	6.40	T	0.16	468	50.6	0.108	72.0	19.1
685 Greenville sandy loam	1–2	5.48	T	0.44	326	74.1	0.227	57.1	29.3
	3–4	4.95	0.7	0.33	204	125.4	0.614	35.6	13.6
	5–6	5.00	0.9	0.41	246	91.2	0.370	42.9	22.1
	7–8	5.15	0.9	0.41	336	57.0	0.169	49.8	24.0
	9–10	6.48	T	0.44	388	45.6	0.117	82.8	23.7

solutions at similar pH values varied considerably. Soil 670, for example, contained no aluminum in solution even at a pH value as low as 4.75; whereas soil 675 contained 3.7 p.p.m. at this pH value and 0.8 p.p.m. at pH 5.03. At pH 4.60 soil 673 contained 1.0 p.p.m. of aluminum, whereas soil 671 at a similar pH value contained nearly three times this concentration. At the uniform pH value of 4.80 it will be noted (table 6) that the concentration of aluminum in the displaced solution of the various soils ranged from zero with three of the soils to 3.1 p.p.m. with soil 675. These differences can probably be explained, in view of the recent interesting work by Joffe and McLean (15), by differences in the kind of anions present in solution. These investigators found aluminum to be soluble at considerably lower degrees of acidity as the nitrate or chloride than as the sulfate or phosphate.

From the yield data given in table 4, and the aluminum data of table 5, it is

possible to study the relation between the concentration of aluminum in solution and the yields of the various crops. If corn is first considered, it will be observed that some relationship seems to exist. This can also be seen from summary table 6. For example, soil 675, which contained the highest concentration of aluminum at pH 4.80, also showed the greatest injury to plant growth whereas soils 671, 672, and 676, having the next highest concentrations of aluminum, showed the next greatest injury. It will be noted, however, that these last three soils which have the same concentration of aluminum, show considerable difference in the extent of the injury to plant growth. It will also be noted

TABLE 6

Summarized data showing the relation between plant growth at pH 4.80 and percentage base saturation, aluminum concentration, and the K/Ca ratio in the soil solution all calculated for the same reaction

SOIL	Al IN DISPLACED SOIL SOLUTION	K/Ca RATIO IN SOIL SOLUTION	BASE SATURATION OF SOIL	GROWTH OF CORN		GROWTH OF SORGHUM		GROWTH OF BARLEY		AVERAGE* RELATIVE YIELDS FOR THE THREE CROPS
				Injury in early growth	Relative yields	Injury in early growth	Relative yields	Injury in early growth	Relative yields	
	p.p.m.		*per cent*							
670	None	0.027	57	No	100	No	100	No	94	98
671	1.1	0.038	32	Yes	62	Yes	66	No	84	71
672	1.1	0.114	10	Yes	80	Yes	16	Yes	25	40
673	0.4	0.060	77	No	87	No	100	No	83	90
674	None	0.115	6	No	84	Yes	65	Yes	37	62
675	3.1	0.339	9	Yes	47	Yes	2	Yes	9	19
676	1.1	0.098	32	No	79	Slight	76	Yes	74	76
678	Not determined	Not determined	20	No	87	No	89	No	100	92
679	None	0.124	43			Very slight	86	Yes	84	85
680	0.5	0.071	18	No	87	No	79	Yes	96	87
683	2.2	0.257	28			No	81	Yes	80	81
684	1.2	0.354	20			Yes	37	Yes	81	59

* Based on yield of best treatment of each series as 100.

that soil 673 at pH 4.60 showed no depression in yield when it contained 1.0 p.p.m. of aluminum in solution. The soils with little or no aluminum in solution gave very little depression in yield of corn at pH 4.80.

As previously mentioned, the yields of sorghum are probably the most significant ones obtained in this study. With this crop there is less correlation between the amounts of aluminum in solution and the yields. Although in general a correlation seems to exist, there are marked discrepancies. For example, the yield of sorghum in pots 3 and 4 of soil 672 containing 1.1 p.p.m. aluminum was only about 16 per cent of that obtained at pH 6.50, whereas that on soil 673 at

an even lower pH value but with practically the same amount of aluminum in solution was just as good as at pH 6.50. A comparison of the results obtained from soil 675 and 683 is also significant in this respect. These are two sandy coastal plain soils, and both have considerable amounts of aluminum in solution. Soil 675 shows very marked injury to the growth of sorghum with as low as 0.8 p.p.m. aluminum, whereas soil 683 with 2.2 p.p.m. and a more acid reaction gave very little depression in yields as compared with the growth at pH 6.50. Other examples of the lack of correlation between yield of sorghum and the concentration of aluminum in the displaced solution are shown in table 6.

The yields of barley, like those of sorghum, show less correlation with the concentration of aluminum in the displaced solution of these soils, than does corn.

Again it may be pointed out, as was done for the pH data, that if only one or a few of the soils of the same origin are considered, a correlation seems to exist; plant growth, in general, decreases as the amount of aluminum in solution increases.

In order to see whether the aluminum concentration in the soil solution, obtained before the sorghum was planted, remained about constant, the soil solutions of a few of the soils were displaced after removal of the crop and their aluminum concentration was determined. Concentrations of 0.9, 1.3, 1.5, 2.7, and 0.6 p.p.m. aluminum were obtained from cultures 3 and 4 of soils 672, 673, 676, 683, and 685. The corresponding values obtained before sorghum was planted were 1.1, 1.0, 1.1, 2.2, and 0.7. Thus it appears that the concentration of aluminum in the displaced solution of these soils remained quite constant during the growth of the sorghum crop.

The question might be raised as to whether aluminum was present in true solution. Although time did not permit a study of this question it would seem from the results obtained by Magistad (23) and by McGeorge (20) that the aluminum found in the displaced solution of soils is in true solution. No indication of colloidal material could be seen in the soil solutions obtained in this study.

It might also be argued that the concentration of aluminum in the soil solution required to be injurious to the growth of crops depends on other factors such as, for example, the concentration of other ions in solution. Burgess and Pember (8), as a result of their studies, concluded that in the presence of large amounts of phosphate, large amounts of aluminum may be absorbed by plants without affecting the growth. If aluminum is the toxic factor and is rendered less toxic by the presence of phosphate, it might be expected that those soils of the present study with high concentrations of phosphate in the soil solution would show little injury at high degrees of acidity even with considerable aluminum present. As will be seen from table 5, this might explain the fact that a concentration of 1.0 p.p.m. of aluminum failed to be injurious to sorghum on soil 673, for this soil has a very high concentration of phosphorus in its displaced solution. The data for the other soils, however, fail to support this viewpoint.

For example, comparisons of soil 672 with 676 and of soil 675 with 683 show that with similar concentrations of phosphorus and of aluminum in solution, the injury to the growth of sorghum was much more marked on soil 672 than on 676 and also on soil 675 than on 683.

Determinations were also made of the total phosphorus absorbed by the plant, since it seemed probable that the concentration of phosphate in solution did not determine the amount of phosphorus absorbed. Again no relation was found between the percentage of phosphorus in the dry plants and the concentration of aluminum in the soil solutions at which plants showed decreased growth.

Comber (9) has suggested that soil organic matter may be active in reducing the toxic effect of excessive aluminum in the soil solution No evidence of such an action, however, is obtained from the organic matter content of these soils as presented in table 1. It will be noted, for example, that soil 672 has a higher content of organic matter than soil 673, and yet soil 672 produced very decided injury to plant growth when containing 1.1 p.p.m. of aluminum in solution, a concentration at which soil 673 gave no injury.

The investigations of McLean and Gilbert (21) with aluminum in culture solutions are of interest. As a result of their studies they classify corn as resistant, sorghum as mediumly sensitive, and barley as sensitive to aluminum. Olsen (26), also working in culture solutions, found that barley of all the plants studied was the only one to show toxicity from aluminum. It is also significant that the concentrations of aluminum required in culture solution for toxicity to plant growth as found by McLean and Gilbert are considerably greater than those found in the displaced solution of the soils of this study on which plant injury from soil acidity was pronounced. These results suggest that toxicity from aluminum may be different in culture solutions than it is in soils, or that the concentration of aluminum required to be toxic is dependent on other factors. This is an added reason for believing that in most acid soils the main factor is not aluminum toxicity. There was some evidence obtained in this study for the belief that the percentage base saturation of soils is such a factor. This point will be considered in connection with the discussion of percentage base saturation of soils as affecting plant growth.

The displaced soil solutions were also analyzed for manganese. No correlation was obtained between the concentration of manganese in solution and plant injury from acidity. Some of the soils showing the least injury to plants had the highest concentration of soluble manganese.

The ratio of calcium to potassium in the soil solution

Pearsall (30) in his studies of the relation between plant distribution and soil acidity suggested that the ratios of the various cations present in the soil solution may be a more important criterion than is the hydrogen-ion concentration of the soils. Cooper and Wilson (11) have a somewhat similar opinion. Very few data are presented, however, in support of this idea. Salisbury (39) raised an objection to Pearsall's theory, namely, that the ratio of potassium to calcium

is not constant but varies with the soil-water ratio, and he cited results by Burgess to show the differences in the potassium-calcium ratio of soil extracts and soil solutions. In this investigation the calcium and potassium were determined in the soil solution; so his objection would not hold. Salisbury believes, however, that "even if we knew the ratios in the soil solution it appears that the proportion in which the ions are absorbed is not determined by the proportions in which they occur as much as by the reaction of the medium, hence we are again forced back on the pH value of the soil."

In table 5 is shown the potassium-calcium ratio of the soil solutions of the various soils at the different pH values. It is first observed that with nearly every soil the ratio of potassium to calcium decreases as the hydrogen-ion concentration increases. This is in accordance with the results presented by Pearsall from the work of Olsen (25) on soil extracts. Among the different cultures of the same soil it will be observed that there seems to be a relationship between the potassium-calcium ratio and plant growth. The point of interest, however, is not so much the ratios found in the soil solutions of the same soil at different pH values, but the ratios for different soils at similar pH values and the relation of these to plant growth. If such a comparison is made it becomes evident that at similar pH values soils differ considerably in their potassium-calcium ratio. This is brought out in table 6 where the ratios are given for all the soils at pH 4.80. It will be noted that at this pH value the basic ratios vary from 0.027 with the Grundy silt loam from Illinois to 0.339 with the Norfolk sandy loam from Alabama. In other words, the former soil has 2.7 per cent as much potassium as calcium in the soil solution, whereas the latter at the same pH value has 33.9 per cent as much potassium as calcium. It will also be observed that there is a fair correlation between the ability of plants to grow on the soils and the potassium-calcium ratio. Two soils prove exceptions to a close correlation, soils 679 and 683. In both these soils a fairly narrow ratio is not accompanied by very poor plant growth. It is possible, however, that magnesium and sodium should also be considered, and that the ratios of sodium and potassium to calcium and magnesium would show a better correlation with plant growth than does the potassium-calcium ratio.

The percentage base saturation of soils as related to the growth of plants on acid soils

The recent excellent studies in Europe and in this country on the base exchange phenomena in soils have led to a better understanding of the nature of soil acidity. It is now generally recognized that soils are acid because of the presence of exchangeable hydrogen. Determinations of the total amount of exchangeable hydrogen of soils, however, cannot serve as a measure of plant response to liming, because most plants can make optimum growth on soils that are not completely neutralized. Since, however, the exchangeable bases are believed to be more readily available to plants than are those in the nonexchangeable form and since soil acidity is believed to be related to calcium

deficiency, it has been proposed that the amount of exchangeable calcium is a criterion for the ability of plants to grow on acid soils. It seems to the writer, however, that the amount of exchangeable calcium, considered by itself, is of much less significance than is its consideration in relation to the exchangeable hydrogen present. If that is true, then one would expect a correlation between the percentage base saturation of soils and plant growth on acid soils.

The percentage base saturation of the soils used in this study was determined; and the values obtained were studied in relation to the growth of plants on these soils. The data are presented in the next to the last column of table 5. As is shown in a paper by Scarseth and the writer (35), the soils vary considerably in their percentage base saturation at similar pH values. This is well shown in table 6 where values are given for the soils at pH 4.80.

There seems to be very little correlation between the depression in yield of corn and the percentage base saturation values. Although the two soils which have the highest percentage base saturation are also those which give the least depression in yields, some soils of low percentage base saturation give very little depression in yields. It must be remembered, however, that with only two of the soils was there much injury to corn from soil acidity. For this reason, the data for this crop are not very conclusive.

Tables 5 and 6 show that with sorghum there is a better, although by no means perfect, correlation between the percentage base saturation of soils and plant growth at low pH values. The most striking example of the correlation is probably for soils 670 and 673. These two soils produce as good growth of sorghum at pH 4.75 and 4.60 respectively as at any higher pH value. For soil 673, at least, these results cannot be explained on the basis of the concentration of aluminum in solution, for this soil contains as much soluble aluminum at pH 4.60 as do other soils which showed decided injury. It will be noted, however, that at these low pH values these two soils are still very highly saturated with bases. All of the other soils which show injury to the growth of sorghum at similar pH values have a much lower degree of saturation. These results, no doubt, explain the fact that soil 670, the Grundy silt loam from the Aledo Experiment Field in Illinois, responds very little to liming in the field although its pH value is between 5.00 and 5.20. Another example of the relation between the percentage base saturation and plant growth is with soils 675 and 683. The difference in yields of these two soils at high degrees of acidity could not be explained on the basis of hydrogen-ion concentration nor of aluminum toxicity. On the basis of percentage base saturation, it will be noted that that the differences in the extent of the injury might be explained.

The yields of barley also show that there is a better correlation between the depression in yields and the percentage base saturation of the soils than between the depression in yields and the concentration of aluminum in the displaced soil solutions. Again there are some exceptions to this correlation. Soils 680 and 678, for example, produced less depression in yields of both the sorghum and the barley crops than would be expected from their percentage base satura-

tion. The lack of a better correlation, however, is not surprising. In the first place, small differences in yields or in the percentage base saturation values cannot be considered significant. Moreover, the injurious action of soil acidity on plant growth is undoubtedly affected by more than one factor.

The correlation that exists between the plant injury to corn on the different soils and the concentration of aluminum in the soil solution, and the indications of some correlation between the aluminum concentration and plant injury to sorghum and barley would suggest that this is probably not incidental, but that soluble aluminum is at least a secondary factor in explaining plant injury on acid soils. Evidence for this is found in these data in the correlation between plant growth and a combination of aluminum concentration and percentage base saturation as compared with the correlation between plant growth and either of these values alone. Where growth is better than would be expected from the percentage base saturation of the soil, the concentration of aluminum in the displaced solution is relatively low; whereas when the growth is poorer than would be expected from the percentage base saturation, the concentration of aluminum in the soil solution is relatively high. For example, soil 675 gave a lower yield than would be expected from the percentage base saturation value as compared with soil 674, but it will be noted that it contains much more aluminum in its displaced solution than does soil 674. Soil 680, on the other hand, gave better yields at pH 4.80 than would be expected from its percentage base saturation when compared with soils 671 and 676, but it contains less aluminum in solution than do the other two soils. It seems, therefore, that the percentage base saturation at which plants are injured on acid soils is affected somewhat by the concentration of aluminum in the displaced solution. Where the concentration of aluminum in the displaced solution is high, plant injury from soil acidity will occur at higher degrees of saturation than when the concentration of aluminum is low. Because of the fact, also, that old weathered soils usually have a lower degree of saturation at given pH values than younger soils, it will be noted that in general the soils of the Piedmont Plateau and Coastal Plain provinces showed a greater injury from acidity at relatively low concentrations of aluminum than did the other soils studied.

GENERAL DISCUSSION

The data presented in this paper show that the percentage base saturation of soils is a better criterion for the growth of sorghum and barley on acid soils than is hydrogen-ion concentration or soluble aluminum. It is also believed from a consideration of these and other data that the percentage base saturation of a soil is of more significance than is the amount of exchangeable hydrogen or the amount of exchangeable calcium present. In this connection the following investigations are of interest. Robinson and Williams (37) found that some soils which gave distinct indications of acidity but did not respond to liming contained 0.2 per cent or more of exchangeable calcium, whereas others that responded to liming contained smaller amounts of exchangeable calcium.

They believe, therefore, that the available calcium is the important factor influencing plant response to liming on the organic soils with which they worked. Rost (38), however, found little correlation between the exchangeable calcium removed by electrodialysis and crop response to lime.

Duley (12) and Fleetwood (13) determined the amount of soil calcium soluble in 0.04*N* carbonated water. This amount of calcium, as was later shown by Robinson and Williams, is smaller than the amount of exchangeable calcium, but it bears a relationship to the exchangeable calcium. Duley and Fleetwood have shown that there is a relationship between the calcium soluble in carbonated water and the response of plants grown on these soils to liming. For all the soils studied, Fleetwood found that those having less than 650 pounds of calcium to the acre gave good responses, whereas those having more than 700 pounds gave poor responses to liming.

It hardly seems possible, however, that if soils varying considerably in texture or total exchange capacity are considered, a correlation would be obtained between the exchangeable or carbonate-water soluble calcium and plant response to liming. For example, many sandy Coastal Plain soils have a total exchange capacity of less than 3 mgm. equivalents per 100 gm. of soil. These soils at pH 6.0 are often between 50 and 60 per cent saturated with bases. This means that the exchangeable calcium is less than 2 mgm. equiv. And yet, most crops on these soils do not respond to liming. Even when they are fully saturated with bases, they contain less than half as much exchangeable calcium as some soils of a heavier texture or of greater total exchange capacity which are very acid and in need of lime. It seems evident, therefore, that the amount of calcium soluble in carbonated water or the amount of exchangeable calcium cannot serve as a safe indication of whether a soil needs to be limed for the successful growth of crops, unless these values are considered in relation to texture or, better still, to the total exchange capacity of soils. It is very probable that the reason Robinson and Williams (37) obtained correlation between exchangeable calcium and plant response to liming, whereas Rost (38) did not, is that Robinson and Williams worked with soils of much the same type, whereas Rost worked with soils of widely different textures and total exchange capacity. Some of the data obtained by Rost can probably be explained on the basis of percentage base saturation. To quote from his statements: "Soil 37 and 22 show nearly the same percentage increase in yield from liming, but the larger amount of lime is removed from the finer textured soil." It is evident that this larger amount of lime removed from the finer textured soil may represent the same or even a lower degree of saturation than does the smaller amount extracted from the coarser soil.

The percentage base saturation viewpoint of the relation between plant growth and soil acidity helps to explain some other facts observed by various investigators. Thus it has been observed by Conner (10), among others, that acid soils which have a high colloidal content are capable of producing good crops even though several tons of lime to the acre are required to bring

them to neutrality. Soils high in colloids have a high total exchange capacity. Therefore, the presence, for example, of 4 mgm. equiv. of hydrogen, which would theoretically require 2 tons of lime an acre for neutralization, leaves a soil that has a total exchange capacity of 20 mgm. equiv. 75 per cent saturated; whereas the presence of that same amount of acid in a soil containing less colloid, such as one having a total exchange capacity of 5 mgm. equiv. would leave that soil only 20 per cent saturated. Moreover, it is interesting to note in this study that there is in general a positive correlation between the total exchange capacity of the soils and their crop producing power. This may partly explain the statement sometimes made that fertile soils are able to withstand greater degrees of acidity than poorer soils. The failure to get response from liming on soils that are quite acid, therefore, may be due to the fact that such soils, although acid, have a high degree of saturation.

The results obtained by Alway and Nygard (2) and by Sewell and Perkins (40) can probably also be explained on the basis of percentage base saturation.

One of the first explanations that was proposed by soil investigators to account for the injurious action of soil acidity on plant growth was that plants in acid soils cannot get sufficient available calcium. The fact, however, that calcium added to acid soils in the form of calcium sulfate did not overcome the injurious action of these soils on plant growth was difficult to explain, for if soluble calcium were present it seemed as if it should be available. Truog (44) in his earlier comprehensive study of soil acidity in its relation to plant growth suggested that the reason calcium sulfate did not prove beneficial on acid soils was that plants needed calcium not so much as a plant nutrient but to neutralize the acids formed within the plant. He concluded, therefore, that the calcium, to be of value, must be present in soils in the carbonate or bicarbonate form. He further concluded that the amounts of calcium which can exist in acid soils in the bicarbonate form are to a large extent determined by the amount and strength of the soil acids present. At the time of his study little was known regarding the base exchange reaction of soils. It is significant, however, that in a large measure his theory is compatible with the percentage base saturation conception. In his more recent studies Truog (45) has emphasized solid phase feeding by which he believes that the calcium in the base exchange compounds may serve the same purpose as that in the carbonate or bicarbonate form.

It may also be that the amount of calcium which can be absorbed by the plant is dependent on other factors such as the relative concentration of calcium to other ions in solution. According to this viewpoint, the fact that calcium sulfate is not beneficial when added to an acid soil could be explained on the basis that it not only adds more calcium to the soil solution but that through the base exchange reaction it liberates considerable hydrogen from the soil complex into the soil solution.

Another factor which probably affects plant growth on acid soils and one that is closely correlated with percentage base saturation is the proportion of

the various exchangeable bases in different soils at similar degrees of base saturation. Although it has been shown by Kelly and Brown (16) and others that the predominant exchangeable base in soils from humid climates is calcium, no one, as far as the writer knows, has studied the relative proportion of the various bases present as soils become progressively more desaturated. It is possible, for example, that two soils may have about the same proportion of the various exchangeable bases at pH 6.5, but that as they become progressively more desaturated the relative proportion of calcium to the other bases maybe quite different. Calcium or magnesium may become a limiting factor much sooner in one of these soils than in the other even though both soils have the same degree of saturation. Moreover, one of the bases may become limiting not only because its total concentration is in itself low, but because it is low in proportion to the other bases present. That such may be the case is indicated by the data on the potassium and calcium content of the soil solutions. Although there was found to be no relationship between plant injury and the amount of calcium in solution, some correlation was obtained between plant injury and the ratio of potassium to calcium in the soil solution. It is possible that the correlation would have been even better if magnesium and sodium had also been determined and if these ratios had been determined for the bases in the exchangeable form.

In this connection the investigations of Cooper and Wilson (11) are of interest. They have proposed the theory that the absorption of ions by plants is influenced by their oxidation-reduction potential. Thus, potassium, which has a high oxidation-reduction potential, would be absorbed much more readily than calcium if present in equivalent concentration. A relatively large amount of potassium compared with calcium, as was found in this study for the soils showing greatest plant injury, would mean that potassium is probably absorbed to the partial exclusion of calcium, even though the latter is present in solution in quite high concentrations.

The recent results obtained by Brown (5) in culture solutions are also of interest. He found that if the molecular ratio of calcium nitrate to potassium di-hydrogen phosphate is decreased from 4:1 to 3:2, a marked reduction in growth is obtained. With the reduction in growth the calcium-potassium ratio in the plant changed from about 1:1 to about 1:4. He also found that increasing the concentration of potassium in the solutions greatly depressed the absorption of magnesium by the plant, even though this element was present in both solutions in the same concentration. The injury to the plants from a relatively high absorption of potassium as compared with calcium and magnesium may be because, as found by True (43), it replaces these elements in the pectates of the middle lamella, causing the cell wall to become more permeable.

These considerations give added reason for believing that the percentage base saturation of soils, and probably the proportion of the various bases present in the exchange complex and in the soil solution, are primary factors which directly influence plant growth on acid soils.

SUMMARY

This investigation was conducted in the greenhouse with 13 soils formed under different climatic conditions and of different degrees of weathering and obtained from several states. Each was brought to five different degrees of acidity by appropriate lime and acid treatments. Crops of corn, sorghum, and barley were grown on the soils. The results of the investigation can be briefly summarized as follows:

The "critical hydrogen-ion concentration" for the growth of crops varied considerably with different soils; therefore, the hydrogen-ion concentration of soils cannot be considered the direct cause of poor plant growth nor the main factor governing plant distribution or response to liming.

The displaced soil solutions of the soils were analyzed for aluminum, calcium, potassium, manganese, and phosphorus.

Aluminum was found present in very small concentrations even in the most acid soils.

The concentration of aluminum in the displaced solution of the different soils at similar pH values varied considerably.

There was some evidence of correlation between the concentration of aluminum in the displaced soil solution and the growth of corn on acid soils but less correlation between the concentration of aluminum and the yield of sorghum and barley. The data are taken to indicate that soluble aluminum is not the primary factor in influencing the growth of acid-sensitive crops on acid soils. Some evidence is given for the belief that it is a secondary factor.

No relation was found between the amount of phosphate in the soil solution and the concentration of aluminum in solution at which plant injury developed.

The concentration of calcium in the soil solution bore no relationship to plant injury on these soils.

A fair correlation was found to exist between the ratio of potassium to calcium in the soil solution and plant injury. As the soils became more acid, the ratio of potassium to calcium decreased. This ratio varied considerably for the different soils at similar pH values.

No relation was found between the concentration of manganese in solution and plant injury from soil acidity.

The determinations of the percentage base saturation of the soils gave interesting results.

Soils varied considerably in their percentage base saturation at similar pH values.

A rather good correlation was found to exist between the percentage base saturation of acid soils and plant injury. Where large amounts of aluminum were present in solution, plant injury seemed to occur at higher degrees of saturation than when aluminum was absent, or present in low concentrations.

The percentage base saturation of soils is believed to be one of the most important factors in determining the growth of plants on acid soils. It is also suggested that differences in the relative proportion of the various bases present in the exchange complex and in the soil solution may be contributing factors to the poor plant growth obtained on acid soils.

REFERENCES

(1) ADAMS, H. R. 1924 Some effects of sulfur on crops and soils. *Soil. Sci* 18: 111–115.

(2) ALWAY, J., AND NYGARD, I. J. 1927 Differentiation between acidity and lime deficiency in the case of peat soils. *Proc. Internatl. Soc. Soil Sci.* (1927) 2: 22–44.

(3) ARRHENIUS, O. 1922 Hydrogen-ion concentration, soil properties and growth of higher plants. *Arkiv. Bot.* 18: 1–54.

(4) BLAIR, A. W., AND PRINCE, A. L. 1922 Studies on the toxic properties of soils. *Soil Sci.* 15: 109–129.

(5) Brown, Duke D. 1929 The effect of different proportions of calcium nitrate and potassium di-hydrogen phosphate on the growth of wheat in sand cultures. *Soil Sci.* 26: 441–446.
(6) Bryan, O. C. 1923 Effect of acid soils on nodule-forming bacteria. *Soil Sci.* 15: 37–40.
(7) Bryan, O. C. 1923 Effect of reaction on growth, nodule formation and calcium content of alfalfa, alsike clover, and red clover. *Soil Sci.* 15: 23–37.
(8) Burgess, P. S., and Pember, F. R. 1923 "Active" aluminum as a factor detrimental to crop production in many acid soils. R. I. Agr. Sta. Bul. 194.
(9) Comber, N. M. 1921 Letter to *Nature* Sept. 29, p. 146.
(10) Conner, S. D. 1921 Liming in its relation to injurious inorganic compounds in the soils. *Jour. Amer. Soc. Agron.* 13: 113–124.
(11) Cooper, H. P., and Wilson, J. K. 1927 Correlation between electromotive series and oxidation potentials and plant and animal nutrition. *Science* 66: 529–31.
(12) Duley, F. L. 1924 Easily soluble calcium of the soil in relation to acidity and returns from liming. *Soil Sci.* 17: 213–228.
(13) Fleetwood, J. R. 1925 Easily soluble calcium of soils as an indicator of their response to liming. *Soil Sci.* 19: 441–458.
(14) Hoagland, D. R. 1919 Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. *Jour. Agr. Res.* 18: 73–117.
(15) Joffe, J. S., and McLean, H. C. 1928 Colloidal behavior of soils and soil fertility: IV. Anion effect on the precipitation reactions and degree of dispersion of aluminum and iron hydroxides. *Soil Sci.* 26: 47–59.
(16) Kelly, W. P., and Brown, S. M. 1924 Replaceable bases in soils. Univ. Calif. Tech. Paper 15.
(17) Line, J. 1926 Aluminum and acid soils. *Jour. Agr. Sci.* 16: 335–364.
(18) Lipman, J. G. 1919 Adjusting the soil reaction to the crop. *Soil Sci.* 7: 181.
(19) Lundell, G. E. F., and Knowles, H. B. 1922 Determination of aluminum as phosphate. *Jour. Indus. and Engin. Chem.* 14: 1136–1137.
(20) McGeorge, W. T. 1924 Iron aluminum, and manganese in the soil solution of Hawaiian soils. *Soil Sci.* 18: 1–11.
(21) McLean, F. T., and Gilbert B. E. 1927 The relative aluminum tolerance of crop plants. *Soil Sci.* 24: 163–175.
(22) McLean, F. T., and Gilbert, B. E. 1928 Aluminum toxicity. *Plant Physiol.* 3: 293–303.
(23) Magistad, O. C. 1925 The aluminum content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci.* 20: 181–226.
(24) Morgan, M. F., Anderson, P. J., and Dorsey, H. 1929 Soil reaction and liming as factors in tobacco production in Connecticut. Conn. Agr. Exp. Sta. Bul. 306.
(25) Olsen, C. 1921 The ecology of *Urtica dioica*. *Jour. Ecology* 9: I.
(26) Olsen, C. 1923 Studies of the H-ion concentration of the soil in its significance to the vegetation. *Compt. Rend. Lab. Carlsberg* 15: 1.
(27) Parker, F. W. 1921 Methods of studying the concentration and composition of the soil solution. *Soil Sci.* 12: 209–232.
(28) Parker, F. W. 1929 Methods for determining the amount and avidity of exchangeable hydrogen in soils. *Proc. First internatl. Cong. Soil Sci.* 2: 167–174.
(29) Parker, F. W., and Fudge, J. F. 1927 Soil phosphorus studies: I. The colorimetric determination of organic and inorganic phosphorus in soil extracts and in the soil solution. *Soil Sci.* 24: 109–118.
(30) Pearsall, W. H. 1926 Soil sourness and soil acidity. *Jour. Ecology* 14: 155–163.
(31) Pierre, W. H. 1927 Buffer capacity of soils and its relation to the development of soil acidity from the use of ammonium sulfate. *Jour. Amer. Soc. Agron.* 19: 332–351.

(32) PIERRE, W. H. 1928 Nitrogenous fertilizers and soil acidity. The use of fertilizer combinations, lime and basic slag in correcting the acidity formed by various nitrogenous fertilizers. *Jour. Amer. Soc. Agron.* 20: 270–279.

(33) PIERRE, W. H., AND FUDGE, J. F. 1928 The adjustment of the reaction of indicator solutions and its importance in determining the hydrogen-ion concentration of slightly buffered solutions. *Jour. Amer. Chem. Soc.* 50: 1254–1262.

(34) PIERRE, W. H., AND PARKER, F. W. 1927 The use of collodion sacks in obtaining clear soil extracts for the determination of the water soluble constituents. *Soil Sci.* 23: 13–32.

(35) PIERRE, W. H., AND SCARSETH, G. D. 1931 The determination of the percentage base saturation of soils and its value in different soils at definite pH values. *Soil Sci.* 31: 99–114.

(36) POWERS, W. L. 1927 The effect of H-ion concentration on the growth of certain plants. *Soil Sci.* 24: 1–8.

(37) ROBINSON, G. W., AND WILLIAMS, R. 1925 Base exchange in relation to the problem of soil acidity. *Trans. Faraday Soc.* 20: 586–593.

(38) ROST, C. O. 1928 Electrodialysis in studies of soil deficiences. *Trans. and Proc. First Internatl. Cong. Soil Sci.* 2: 334–341.

(39) SALISBURY, E. J. 1925 The incidence of species in relation to soil reaction. *Journal Ecology* 13: 149.

(40) SEWELL, M. C., AND PERKINS, A. T. 1927 Base exchange studies of Kansas soils correlated with crop performance and fertilizer practice. *Trans. and Proc. First Internatl. Cong. Soil Sci.* 2: 175–184.

(41) TIDMORE, J. W. 1927 Studies of the cobalt-nitrite colorimetric method of determining small amounts of potassium. Not published.

(42) TRENEL, M. 1926 Is soil reaction of importance for the practical agriculturist? Abs. in *Internatl. Rev. Sci. and Pract. Agr. (Rome)* 4: 809.

(43) TRUE, R. H. 1922 The significance of calcium for higher green plants. *Science* 55: 1–6.

(44) TRUOG, E. 1918 Soil Acidity: Its relation to the growth of plants. *Soil Sci.* 5: 169–195

(45) TRUOG, E. 1928 How plants feed. *Trans and Proc. First Internatl. Cong. Soil Sci.* 4: 628–636.

(46) WHERRY, E. T. 1927 Divergent soil reaction preferences of related plants. *Ecology* 8: 197–206.

(47) YOE, J. H., AND HILL, W. L. 1927 An investigation of the reaction of aluminum with the ammonium salt of aurinitricarboxylic acid under different experimental conditions, and its application to the colorimetric determination of aluminum in water. *Jour. Amer. Chem. Soc.* 49: 2395–2407.

CHANGES IN THE AVAILABILITY OF PHOSPHORUS IN IRRIGATED RICE SOILS[1]

R. P. BARTHOLOMEW[2]

Received for publication May 12, 1930

Phosphatic fertilization of rice, as a rule, has not been profitable in Arkansas on soils which are known to be deficient in available phosphorus. The increased yields, if any, are not large enough to repay the cost of the fertilizer. Why should rice fail to respond to phosphatic fertilizers on soils which produced marked increases with applications of phosphorus-carrying fertilizers to other crops, such as soybeans?

A hypothesis commonly offered by rice growers to explain the condition mentioned, is that phosphatic fertilizers stimulate weed growth to such an extent that the weeds can not be controlled by irrigation. As a consequence, a considerable amount of the plant nutrients are used up by the weeds and no increased yields of rice are obtained. This hypothesis, although logical, is based largely on field observations and does not consider the abnormal conditions which develop in the soil after the rice is irrigated. Neither does it consider the fact that phosphatic fertilizers on rice have been reported as profitable in some instances in other countries (6) and (12). A survey of the conditions under which rice is grown suggests that a number of factors other than weed growth may be active in affecting the availability of phosphorus in rice soils.

Rice in Arkansas is grown in a region underlain by limestone rocks. As a result the water from wells used for irrigation may contain considerable amounts of calcium which could cause phosphorus in the soils to revert to insoluble forms. These are unavailable for rice, which, as has been previously shown (1), is a very poor feeder on insoluble phosphates. The development of anaerobic conditions after irrigation might cause some of the available phosphorus to be changed to forms which are unavailable for rice.

The investigation reported herein was started in an effort to study some of the factors which may affect the availability of phosphorus under the conditions of rice production and to determine the manner in which those factors act.

[1] Contribution from the department of agronomy, University of Arkansas. Published with approval of the director. Research Paper No. 223 Journal Series, University of Arkansas.

[2] Assistant agronomist. The writer wishes to express his appreciation to A. H. Prince of Stuttgart and R. D. Setzler, assistant at the Arkansas Rice Experiment Station, for their valuable assistance in obtaining samples of soil and irrigation water.

PLAN OF EXPERIMENT

The work was planned to determine by chemical analyses the reaction, the calcium content, and the iron and aluminum content of water from wells used for irrigation purposes; to determine by chemical analyses the reaction of surface and subsoils devoted to rice production; and to study under controlled conditions the effect of irrigation upon the availability of phosphorus in rice soils which had received different fertilizer treatments.

Calcium, iron, and aluminum content of irrigation waters

A 2½-liter sample of water was taken as it came from the pump from each of several different wells in the rice area when they were pumping water for the irrigation of rice fields. The samples were brought to the laboratory and their calcium, iron, and aluminum content and reaction were determined. Iron and aluminum were not determined separately, inasmuch as their effect on soluble phosphorus compounds would be similar. In addition, the reaction of water from several wells from which no samples were taken for analyses was determined colorimetrically. The results of the analyses are given in table 1.

The reaction of all samples was either neutral or alkaline. The suggestion, given by the alkaline reaction of the water, that they might contain considerable amounts of basic material in solution is proved by the results of the chemical analyses. The amounts of calcium, although variable, are fairly large. The results are very consistent and agree with results of analyses made by the United States Geological Survey (13). Their results showed the calcium content to vary from 22 to 112 p.p.m., whereas the iron content was from 0.55 to 6.56 p.p.m. The calcium content varied from 20.3 p.p.m. to 74.8 p.p.m., although most of the results were near the latter figure. The amounts of iron and aluminum are likewise variable, but are present in appreciable amounts, varying from 6.1 to 12.8 p.p.m.

The full significance of the presence of calcium, iron, and aluminum in the irrigation water is seen when the amounts added annually in the irrigation water are calculated. According to the preliminary results of investigations by Carter and Clayton (3), the net duty of water in the rice area is 22.3 inches. Because of an impervious subsoil, vary little of this water is lost by seepage. It is largely removed by transpiration of the plants and evaporation from the surface of the water. As a result, practically all of the basic material applied in the irrigation water is deposited in the soil. The amounts applied annually are given in table 2.

In all but two of the samples, more than 300 pounds of calcium, free to combine with either insoluble soil acids or the more soluble forms, such as phosphoric and sulfuric acids, are added annually. In addition, from 30 to 60 pounds of soluble iron and aluminum are added. Thus rice soils, cropped successively to rice for a period of years because of the expense involved in

establishing irrigation systems, receive an enormous amount of soluble basic materials in a relatively short time.

These materials added in the irrigation water could greatly decrease the supply of available phosphorus. A residue of 194 pounds of calcium would

TABLE 1

Analyses of well waters used for irrigation of rice fields

SAMPLE NUMBER	pH	Ca IN WATER	Fe_2O_3 AND Al_2O_3 IN WATER
		p.p.m.	*p.p.m.*
1	7.5		
2	7.5		
3	7.0		
4	7.5		
5	7.5		
6	7.5		
7		24.5	
8	7.2	66.8	11.6
9	7.3	20.3	12.8
10	7.3	74.8	12.4
11	7.4	66.2	12.0
12	7.2	70.0	8.5
13	7.4	66.4	6.1
14	7.0	51.4	6.2
15	7.2	66.8	8.9
16	7.3	72.3	6.4
17	7.4	70.9	11.6

TABLE 2

Approximate amounts of calcium and iron and aluminum added to the soil annually by irrigation water

SAMPLE NUMBER	Ca ADDED PER ACRE	Fe_2O_3 AND Al_2O_3 ADDED PER ACRE
	pounds	*pounds*
8	325	56
9	98	62
10	363	60
11	320	58
12	339	41
13	321	29
14	249	30
15	323	43
16	350	31
17	343	53

have the ability to revert 150 pounds of available phosphorus in the soil, if it existed as the monocalcium salt. This is considerably more than most soils contain and is more than the amount contained in one ton of superphosphate. This does not necessarily mean that such a reaction takes place, but merely

illustrates the enormous fixing power of the material added to the soil each year. On the other hand, this basic material might be used to neutralize soil acids. However, even if the added basic materials were utilized largely in neutralizing both soluble and insoluble soil acids they would still increase the amount of replaceable base in the soil. The ultimate effect on the soluble phosphorus compounds would be the same. This is in excellent agreement with the results of Metzger (7), who has shown that continued irrigation has greatly increased the amount of replaceable calcium in the surface soil.

Effect of irrigation on soil reaction

The annual addition of such large amounts of basic materials to soils should result in the soils becoming more alkaline. Although determinations of the reaction of soils some years after irrigation without a definite knowledge of the initial reaction of the soil might give misleading results, the conditions in the rice area of Arkansas are such that the general trend of such changes may be obtained. The soil is a rather heavy silt loam underlain by an impervious subsoil. The subsoil is so impervious that very little of the irrigation water is lost by percolation. As a result, most of the materials carried by the irrigation water are deposited in the surface layer. The surface soil, therefore, should become more alkaline than the subsoil. The reactions of a number of surface and subsoils taken from farms within a radius of 20 miles in the rice district are given in table 3.

TABLE 3

Effect of irrigation on the reaction of rice soils

SAMPLE NUMBER	SURFACE SOIL pH	SUBSOIL pH	SAMPLE NUMBER	SURFACE SOIL pH	SUBSOIL pH
1	5.6	5.2	17	7.0	6.8
2	5.9	*	18	5.2	5.3
3	6.2	*	19	6.4	6.6
4	6.7	5.9	20	6 8	5.9
5	7.4	*	21	6.7	5.6
6	6.1	*	22	6.8	6.5
7	6.9	*	23	6.6	5.8
8	6.3	6.0	24	7.1	6.3
9	7.4	6.6	25	6.7	6.0
10	7.0	6.3	26	6.4	5.8
11	5.5	5.6	27	6.6	6.1
12	6 1	5.5	28	7.0	6.5
13	7.1	6.2	29	6.6	5.4
14	6.1	5.8	30	6.7	5.7
15	7.0	5.3	31	6.5	4.9
16	6.4	6.0			

* Subsoil not sampled.

The surface soil was taken from the surface 6 inches and the subsoil from the

12 to 18-inch layer. Sample 11 has been in rice only one year. Samples 18 and 19, from virgin soil, illustrate the conditions generally found in virgin soils of this type, viz., the reaction of the surface and subsoil are almost the same. The results show very clearly the tendency of irrigation to make the surface more alkaline. In some cases, 4, 9, 10, 13, 15, 17, 23, and 28, the soil acids have been completely neutralized whereas in many of the others it has been almost all neutralized. While neutralization was taking place, the abundance of basic materials was undoubtedly reverting some of the phosphorus in the soil and making it unavailable for the growth of rice.

Water-soluble phosphorus in rice soils

To make normal growth, all plants need a certain amount of phosphorus. If the amount coming from the soil is insufficient for normal needs, the amount of growth is reduced. With the addition of so much basic material in the irrigation water, it is important that the amount of phosphorus in the soil solution be known. A knowledge of the ability of the soil to maintain that concentration is of equal importance.

Analyses for phosphorus by the ceruleo-molybdate method of Deniges (5), as modified by Parker and Fudge (9), in the displaced soil solution showed it to contain 0.75 p.p.m. of total PO_4, of which 0.3 p.p.m. was in the inorganic form, and, according to Pierre and Parker (11), is available to plants. From our present knowledge of phosphorus requirements of plants (8) 0.3 p.p.m. inorganic phosphorus would enable plants to make normal growth provided that concentration was maintained.

The soil solution had a pH of 7.2, indicating the presence of considerable basic material. Qualitative analyses showed the presence of considerable calcium.

In order to test the ability of the soil to maintain its original supply of available phosphorus, an experiment was performed to detemine the rate at which renewal of phosphorus in the soil solution would take place. The amount of inorganic phosphorus coming into solution during various intervals was determined by the collodion bag method (10). Thirty grams of soil was placed in the collodion bags and 50 cc. of water added. One hundred cubic centimeters of distilled water was added to the outside. At each sampling, the 100-cc. portion on the outside was removed for analysis and another 100 cc. of distilled water added to the flask. The inorganic phosphorus was determined, as given in the foregoing and the pH was determined colorimetrically. Because of the possibility that the abundant bacterial growth which developed inside the collodion bags might have affected the availability of the phosphorus, a second set was run in which an effort was made to control bacterial growth by the addition of a few drops of toluene. The results of both experiments are given in table 4.

The results show that while the concentration of inorganic phosphorus in the soil extract is low, 0.13 p.p.m., the soil is able to maintain the amount in

solution at a rather uniform level. On the other hand, factors such as the rapid development of microorganisms may cause a very rapid depletion of the inorganic phosphorus in solution. This occurred after 8 days, where no toluene was added, and at the end of 10 days when toluene was added at the beginning of the experiment. Constant removing and renewal of solution had probably depleted the supply of toluene added at the beginning of the experiment. The microorganisms developed so rapidly that in a few days they caused the liquid outside of the collodion bags to become cloudy.

There is a suggestion in the results of the second experiment of the repression caused by the basic materials added in the irrigation waters. There is a gradual decrease in the water-soluble phosphorus for a time, followed by an

TABLE 4

Inorganic PO_4 in successive diffusates from 1:5 ratio of rice soil and water

TIME	INORGANIC PO_4 IN DIFFUSATE	PO_4 DISSOLVED IN INTERVALS BETWEEN SAMPLING	pH
hours	*p.p.m.*	*p.p.m.*	
Without toluene			
24	0.13	0.13	6.7
48	0.13	0.09	...
84	0.08	0.04	...
108	0.08	0.05	6.0
132	0.08	0.05	...
180	Trace	—	6.4
With toluene			
24	0.08	0.08	6.5
48	0.07	0.05	6.7
72	0.06	0.04	6.8
120	0.09	0.07	7.2
144	0.08	0.05	7.2
168	0.10	0.08	7.3
240	Trace	—	7.2

increase as the extraction continued. Accompanying these changes, there is a gradual increase in pH from 6.5 to 7.3, presumably because of basic material coming into solution. This is in agreement with Burd and Martin (2), who state that as the cations are removed from a soil more phosphorus will go into solution.

The relatively small amount of inorganic phosphorus found in the soil solution readily accounts for the marked response of other crops than rice where applications of soluble phosphatic fertilizers are made to these soils.

Changes in availability of phosphorus produced by irrigation

In addition to the effects given in the preceding experiments, there is also the possibility that the anaerobic conditions caused by the irrigation of the

TABLE 5

Amounts of PO_4 in 1:5 water extracts of soils with the fertilizer and irrigation treatments indicated

TREATMENT		PO_4 IN EXTRACT								
		May 6			June 8			July 22		
Fertilizer	Irrigation	Inorganic	Organic	Total	Inorganic	Organic	Total	Inorganic	Organic	Total
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
None	None	0.06	0.30	0.36	Trace	0.14	0.14	None	0.14	0.14
None	Flooded with distilled water	Trace	0.46	0.46	Trace	0.21	0.21	None	0.55	0.55
250 pounds $CaH_4(PO_4)_2$	Flooded with distilled water	0.09	0.43	0.52	0.11	0.17	0.28	Trace	0.64	0.64
200 pounds $(NH_4)_2SO_4$	Flooded with distilled water	Trace	0.38	0.38	Trace	0.32	0.32	None	0.44	0.44
250 pounds $CaH_4(PO_4)_2$	Flooded with calcium hydroxide* solution	0.07	0.47	0.54	Trace	0.35	0.35	Trace	0.74	0.74
None	Flooded with calcium hydroxide solution	Trace	0.61	0.61	Trace	0.28	0.28	None	0.46	0.46

* Calcium equivalent to 300 pounds an acre was added in first four applications and distilled water was used for later irrigations.

rice fields may produce a change in the kind or amount of water-soluble phosphorus in the soil. A series of jars containing equal amounts of soil, but receiving different treatments, were started in the greenhouse to study the effect of irrigation on the amount and forms of water-soluble phosphorus in the soil. Clear solutions of 1:5 water extracts were obtained by the collodion bag method. They were analyzed colorimetrically for organic, inorganic, and total water-soluble PO_4. The fertilizers were added and the soils flooded April 5, 1929. The treatments and results of the analyses of different samplings are given in table 5.

Flooding has apparently caused relatively rapid fixation of the soluble inorganic phosphorus in the soil solution. Only one month of flooding has caused the disappearance of nearly all of the available phosphorus. This has taken place even where mono-calcium phosphate was added at the rate of 250 pounds an acre. This was probably due to reversion of the phosphate.

The effect of the increase of basic material in solution is also seen in the change in pH from the first and second sampling. At the first sampling, the unflooded check had a pH of 6.1 whereas the others had a pH of 6.3. On June 8, the acidity of the unflooded check had increased to pH 5.72, whereas all the others decreased to between pH 6.92 and pH 7.18. This change, except in the two treatments receiving calcium hydroxide in the irrigation water, could be attributed only to the basic material coming into the solution.

It is also shown that part of the phosphorus may have been converted to an organic form. As a result of the suggestion given from the results of the collodion bag experiments an effort was made to observe the changes, if any, in biological activities by means of the Conn direct staining method (4). Although differences in type of organisms were undoubtedly present, they were not outstanding enough to be observed by the writer. The amount of organic phosphorus in the extract for the July 22 analysis shows a decided increase in all of the flooded treatments, further supporting the idea that inorganic phosphorus may be transformed to organic phosphorus. This also may be a factor concerned in the phosphatic fertilization of rice.

The completeness of reversion is shown by the following experiment. After the July 22 sampling, the moisture content was reduced by normal evaporation to 10 to 18 per cent and maintained in that range for six months. At the end of the time, no more than a trace of water-soluble inorganic phosphorus was found in any jar.

DISCUSSION OF RESULTS

The maintenance of an adequate supply of plant-food constituents for good yields of crops is the primary object of fertilizer practices. Any factors which may affect the supply of available plant-food will naturally affect the practices operating in the fertilization of crops.

Admittedly the application of phosphatic fertilizers to soils deficient in phosphorus will stimulate weed growth just as it does crop growth. If the

weed growth is not controlled, it is self evident that there will be competition between weeds and rice, which is a poor forager for available phosphorus, with the stronger feeder getting the major portion of the available phosphorus.

On the other hand, it seems quite evident that if other crops can compete successfully on the same soil with weeds and make profitable increased yields with applications of phosphatic fertilizer, then poor returns from the use of phosphatic fertilizers on rice must be linked in some way with this particular crop.

The application of large amounts of basic materials annually in the irrigation water used on rice has resulted in reducing the hydrogen-ion concentration of soils very much. In some instances, the change has been from strongly acid to a decidedly alkaline reaction. The result has been that phosphorus has reverted to slightly soluble forms and is only slowly available to rice, which is a weak feeder on insoluble phosphates.

At the same time, irrigation of the soils causes an increase in replaceable bases in the soil, thus causing reversion of the more soluble phosphate. As a result, a few weeks after irrigation, the amount of inorganic phosphorus in the soil may be practically nil. Reversion takes place even when soluble phosphatic fertilizers are added to the soil.

The anaerobic conditions produced by the irrigation may cause a change in the type of inorganic forms of water-soluble phosphorus, which are available to plants, to organic forms which are not available to plants.

The action of most factors affecting soil conditions in the production of rice is to decrease the solubility of phosphatic fertilizers. From the results of these experiments, it does not seem advisable to recommend the use of phosphatic fertilizers where the land is irrigated with calcareous water, until some means is found to keep the phosphorus in solution.

SUMMARY

Studies were made under field conditions to determine the effect of irrigation on the pH of rice soils. The calcium content, iron and aluminum content, and pH of well water used for irrigation puposes were found by analysis. The effect of irrigation on the availability of phosphorus in soils receiving different fertilizer treatments was studied. The results of the investigation may be summarized as follows:

Water used for the irrigation of rice in Arkansas contains large amounts of calcium, largely in the bicarbonate form.

From 6 to 12 p.p.m. of soluble iron and aluminum were found in the samples analyzed.

The amounts of soluble calcium and iron and aluminum added annually in the irrigation water could cause reversion of large amounts of soluble phosphorus.

Continued irrigation with calcareous well water has made the surface soil in the rice area decidedly more alkaline than the subsoil.

Irrigation caused a descrease in the water-soluble inorganic phosphate. This was partially due to reversion of the phosphates.

The increase in the organic water-soluble phosphorus three months after irrigation may be due to activities of bacteria under anaerobic conditions.

Phosphatic fertilizers, according to the results of these experiments, should not be recommended when calcareous water is used for irrigation.

REFERENCES

(1) Bartholomew, R. P. 1928 The unavailability of phosphorus in rock phosphate to some southern crops. Jour. Amer. Soc. Agron. 20: 913–920.

(2) Burd, J. S., and Martin, J. C. 1923 Phosphate behavior in soils. *Science* 58: 227–228.

(3) Carter, Deane G., and Clayton, B. S. Private communication from the department of agricultural engineering, University of Arkansas.

(4) Conn, H. J. 1928 On the microscopic method of studying bacteria in soil. *Soil Sci.* 26: 257–259.

(5) Deniges, G. 1920 Reaction de coloration extremement sensible des phosphates at des arseniates. Ses applications. *Compt. Rend. Acad. Sci.* (*Paris*) 171: 802–804.

(6) McKerral, A. 1916 Report of Hmawbi Agricultural Experiment Station for the year 1915–16. Dept. Agr. Burma, Rpt. Hmawbi Agr. Exp. Sta. 1915–16.

(7) Metzger, W. H. 1929 The effect of moisture content and cropping on exchangeable calcium and magnesium with particular reference to rice soil. *Soil Sci.* 27: 303–318.

(8) Parker, F. W. 1927 Plant growth and the absorption from culture solutions of different phosphate concentrations. *Soil Sci.* 24: 129–146.

(9) Parker, F. W., and Fudge, J. F. 1927 The colorimetric determination of organic and inorganic phosphorus in soil extracts and in the soil solution. *Soil. Sci.* 24: 109–117.

(10) Pierre, W. H., and Parker, F. W. 1927 The use of collodion sacks in obtaining clear soil extracts for the determination of the water-soluble constituents of the soil. *Soil Sci.* 23: 13–32.

(11) Pierre, W. H., and Parker, F. W. 1927 Soil phosphorus studies: III. The concentration of organic and inorganic phosphorus in the soil solution and the availability of organic phosphorus to plants. *Soil Sci.* 24: 119–128.

(12) Sherrad, G. 1915 Report of field crops work at the Bankipoor Agricultural Station 1915–16. Rpt. Dept. Agr. Bihar and Orissa 1915–16.

(13) United States Geological Survey. Private communication from D. G. Thompson, geologist in the United States Geological Survey.

THE INFLUENCE OF COMBINED NITROGEN ON GROWTH AND NITROGEN FIXATION BY AZOTOBACTER

JAMES E. FULLER AND LEO F. RETTGER

Yale University

Received for publication May 6, 1930

It is commonly assumed that available combined nitrogen in the substrate will inhibit nitrogen fixation by Azotobacter. Mockeridge (23) says: "It is a well known fact that when supplied with soluble nitrogen, Azotobacter does not fix atmospheric nitrogen until the available nitrogen has been consumed."

This assumption is apparently accepted by many investigators in the field of Azotobacter physiology. The evidence in the literature, however, hardly seems adequate to justify the unqualified acceptance of the idea that the presence of such nitrogenous materials in the soil or other substrate will necessarily depress nitrogen fixation by Azotobacter. In fact, some of the investigations reported point in the opposite direction.

Krainski (18) observed that nitrogen fixation is more active in rich or well-fertilized soils than in poor soils. Löhnis and Green (20) employed a synthetic medium to which they added manure, straw, and peat. They inoculated this medium with Azotobacter and observed that nitrogen fixation was increased. Greaves and Carter (9) found that the treatment of soil with stable manure under field conditions had a beneficial effect upon the nitrogen-fixing power of the soil. Large quantities of manure did not prove as beneficial as smaller quantities.

Richards (26) investigated the effect of garden soil when added to fresh horse or cow manure. He found that the nitrogen content of the mix increased during incubation, and isolated Azotobacter and *B. lactis-aerogenes* from the cultures. Greaves and Nelson (10) state that ". . . . commercial fertilizers may discourage the beneficial bacteria, including nitrogen-fixing organisms, to such an extent that they cease to gather nitrogen from the air, whereas the use of farmyard manure may encourage them." They further suggest that manure carries phosphorus, potassium, and other food to the soil. These materials may be as much responsible as the nitrogen for any stimulating influence on nitrogen fixation.

Beijerinck and Van Delden (3) showed that Azotobacter has the power to attack nitrates, converting them to ammonia. Lipman (19), in a study of *Azotobacter vinelandii*, tried the effect of potassium nitrate, peptone and ammonium chloride. He found that available nitrogen reduced the fixation of atmospheric nitrogen. Stoklasa (30), Stranak (31), and Heinze (12) showed that small quantities of nitrates stimulated nitrogen fixation by Azotobacter.

Hills (13) tried the effects of sodium, potassium, and calcium nitrates on soil cultures of Azotobacter. He employed 150 gm. dry weight of soil as the medium and added the nitrates in quantities of from 10 to 300 mgm. to each culture, with 1 per cent mannitol as a source of energy. The numbers of Azotobacter increased in cultures containing up to 100 mgm. of sodium, potassium, and ammonium nitrates, and 50 mgm. of calcium nitrate. Maximum nitrogen fixation was observed with 100 mgm. of sodium and potassium nitrate in the case of one Azotobacter strain, and with 150 mgm. of each with another strain of the organism. In every instance the use of calcium nitrate caused a marked decrease in the amount of nitrogen fixed.

Hills found, further, that sterilized soil containing sodium nitrate in quantities of 50 and

150 mgm. to 100 gm. of soil showed at least three times as much nitrogen fixation as the controls. There was not much difference between the two concentrations. In un-sterilized soil 50 mgm. gave a marked increase in nitrogen fixed, whereas with 150 mgm. almost twice as much was formed.

Tests in sterilized soil showed a marked increase in nitrogen fixation with 50 mgm. of calcium nitrate in 100 gm. of dry soil, but not quite as much as with 200 mgm., the proportion being 7.8 to 9.8 mgm. of nitrogen fixed. In un-sterilized soil the results were similar when 50 mgm. of the nitrate were added, whereas 200 mgm. showed a fixation of 12.8 mgm. of nitrogen in 100 gm. of soil. The nitrogen was found as organic nitrogen. Any loss resulting from the attack on the nitrate is made up by the fixation of free atmospheric nitrogen.

The percentage increase in the numbers of viable Azotobacter in these experiments was about 10 times as great as the maximum nitrogen increase. Hence, the results show that the physiological efficiency of the Azotobacter, in so far as fixation of atmospheric nitrogen is concerned, is actually decreased to a marked degree.

Bonazzi (4) found that the addition of calcium nitrate not only resulted in the inhibition of nitrogen fixation by the organism, but that there was some loss of the nitrogen from the nitrate incorporated in the medium.

Zoond (37) observed that nitrogen fixation was depressed when potassium nitrate, tyrosine and Bacto-peptone respectively were added to a medium in quantity sufficient to give a nitrogen concentration of more than 0.003 per cent. Maximum fixation occurred in a concentration of 3 mgm. of nitrogen in 100 cc. of solution. The drop in nitrogen fixation was rapid as increasing quantities of the substance were added. The investigator suggests that the apparent stimulus resulting from small quantities of nitrogenous materials may be due to the law of concentration of ions, as observed by Winslow and Falk (36) in the use of increasing quantities of sodium chloride and calcium chloride on the rate of dying of *B. coli*. Zoond adds his suggestion to that of Bonazzi, that nitrogen fixation is a reserve power resorted to by Azotobacter only in case of nitrogen starvation.

McBeth (21) investigated the effect of 0.1 per cent ammonium sulfate on the nitrogen-fixing activities of *Azotobacter chroococcum* and *A. beijerinckii*. He concluded that the presence of ammonium sulfate stimulates nitrogen fixation by the organisms. He made no mention of the amount of growth. He conducted qualitative, but not quantitative, tests for ammonia.

Reed and Williams (25) tried the effects of various organic nitrogen compounds, including caffeine, trimethylamine, asparagine, creatinine, xanthine, hypoxanthine, urea, formamide, glycocoll, allantoine, guanidine carbonate, and skatole. They found that nitrogen fixation was only slightly affected by most of the compounds investigated. Guanidine and skatole were toxic, whereas caffeine appeared to stimulate the growth of Azotobacter. Urea, glycocoll, formamide, and allantoine were especially active in depressing nitrogen fixation, because the authors suggest, of their satisfying the nitrogen demands of Azotobacter, rather than because of any toxic effect. The simpler substances appeared to exercise the most depressing effect. This might be expected because they probably are the more easily utilized.

It seems logical that, as proteins are decomposed in the soil, the degradation products, including amino acids, should be present in definite but varying quantities. Amino-acid nitrogen was first demonstrated in soil by Warrington (34) and later by Sestini (29). Dojerinko (7) made quantitative estimates of the amounts of monamino and amido nitrogen in humic acid extracted from Russian soil. Jodidi (14) first demonstrated the presence of diamino acids. Schreiner and Skinner (28) reported the following organic nitrogen compounds in soil: arginine, adenine, cytosine, choline, creatine, creatinine, histidine, hypoxanthine, nucleic acid, guanine, and trimethylamine.

Jodidi (16) states that nitrogen in its initial stages may be found in considerable quantities in soil. The amino acids and acid amides cannot accumulate to any extent because they are easily ammonified. The ammonia and nitrites cannot accumulate because they are readily

oxidized to nitrates which are utilized by plants or leached out of the soil if it is fallowed. He (15) analyzed a series of soil samples which showed an ammonia content of 0.0015 per cent or less and a nitric content of less than 0.01 per cent. Oven-dried soils contained 0.257 per cent or less of total nitrogen, 0.0015 per cent or less of ammonia, and 0.096 per cent or less of acid amides.

Headden (11) reported the following analyses of Colorado soils: protein nitrogen, 0.07 to 0.08 per cent; ammonia nitrogen, near 0.01 per cent; amino nitrogen, average about 0.04 per cent; nitric nitrogen, 0.05 per cent or lower; total nitrogen, mostly below 0.2 per cent with a few samples showing as much as 0.3 per cent.

Neidig and Snyder (24) found that Idaho soils contained from 0.152 to 0.886 per cent total nitrogen. Russell (27) states that the total nitrogen of arable soils is usually about 0.15 per cent, and that of pasture lands 0.03 per cent. The ammonia content of heavily dunged areas is 0.001 per cent. Rich garden soils contain 0.006 per cent of nitrates, and arable soil 0.002 per cent.

PRESENT INVESTIGATION[1]

Methods

Four strains of *Azotobacter chroococcum* were employed. Three of these were stock laboratory strains of the organism, and are designated in the tables as A1, A3, and A4. All were able to fix substantial quantities of free atmospheric nitrogen when cultivated in a medium free from combined nitrogen, A4 being the least active in this respect. The fourth strain, designated as Am, was freshly isolated from soil. It fixed free atmospheric nitrogen more actively than the other three strains. Although it exhibited the characteristic morphology of Azotobacter, it did not produce as much pigment in cultures as the three stock strains.

Experimental work in nitrogen fixation is frequently criticised on the ground that the Azotobacter cultures used have been carried in the laboratory on media containing no combined nitrogen until they have been trained to fix free atmospheric nitrogen. This objection is eliminated partially in this study by the use of strain Am, which was re-isolated several times during the progress of the work.

Ashby's (1) medium was employed for all of the experiments. Its composition is as follows:

KH_2PO_4	0.2 gm.
$MgSO_4$	0.2 gm.
NaCl	0.2 gm.
$CaSO_4$	0.1 gm.
$CaCO_3$	5.0 gm.
Mannitol	12.0 gm.
Water	1,000 cc.

The KH_2PO_4 is neutralized to phenolphthalein with 10 per cent NaOH before it is added to the rest of the medium. If a solid medium is desired,

[1] For a more detailed presentation of some phases of the investigation, the reader is referred to the doctorate dissertation of the senior author in the Yale University library.

1.5 per cent agar is added. Ashby advocated starting the cultures of Azotobacter on an agar base in the culture flasks or bottles, and after three days' incubation at the desired temperature, adding the nutrient solution in desired quantities. This method insures better seeding of the Azotobacter than can be obtained otherwise. All of the media were sterilized at 15 pounds for 20 minutes.

In the following experiments, 10-cc. quantities of Ashby's agar were placed in 200-cc. Blake culture bottles and the bottles set on edge after sterilization. An inoculum was prepared by making a suspension of a week-old culture of Azotobacter in physiological saline and adjusting the density to 5.0 in the McFarland (22) nephelometer scale. By means of a sterile pipette, 1 cc. of the suspension was transferred to the agar in a Blake bottle. After three days incubation at 28°C., 50 cc. of Ashby's nutrient solution was added.

In the experiments in which various forms of combined nitrogen were used the desired amount of the nitrogenous substance was added to both the solid and fluid forms of the Ashby medium.

Preliminary experiments determined the most favorable time and temperature for incubation to be 21 days at 28° to 30°C. The quantity of inoculum was found to be unimportant, within reasonable limits, as long as a sufficient seeding was obtained to give a satisfactory growth. The nitrogen content of the inoculum employed was not sufficient to be measurable by the Kjeldahl method and consequently did not influence the total nitrogen determinations in the cultures after incubation.

After 21 days' incubation at 28°C., determinations were made of the amount of growth, ammonia and amino acid nitrogen, and total nitrogen.

The estimates of growth were checked by a direct microscopic examination of the cultures, using the technic advised by Conn (6). Rose Bengal was employed as a stain. Direct plate examinations of the cultures for numerical determination of the bacteria present did not prove sufficiently consistent to be satisfactory, probably because of the mucous-like consistency of the Azotobacter growth.

Ammonia determinations were made with the Van Slyke and Cullen modification of the Folin air current method. Amino acid nitrogen was determined by Brown's (5) modification of the technic of Henrique and Sørensen. The estimations of ammonia and amino acid nitrogen in the cultures were begun with the hope that they could be used as measures of the nitrogen fixed by Azotobacter. These proved to be of no value in that way because no ammonia or amino acid nitrogen could be detected in cultures of the organism grown in a solution containing no combined nitrogen. In the experiments on the effect of combined nitrogen, however, both the amino acid nitrogen and ammonia tests were useful in estimating the activity of Azotobacter in attacking the nitrogen compounds.

Total nitrogen was determined by means of the Kjeldahl method. Five per cent copper sulfate was used as a catalyzer. Distillations were made into

$N/14$ sulfuric acid which was titrated back with $N/14$ sodium hydroxide, Congo Red being used as an indicator.

Experiments were conducted to determine the possible toxic properties of the nitrogen compounds towards Azotobacter. For these tests Ashby's agar containing the desired concentration of combined nitrogen was inoculated with Azotobacter and plates were poured in the usual way. The plates were incubated for one week at 28°C.

Does Azotobacter fix free atmospheric nitrogen in a definitely controlled atmosphere containing no combined nitrogen?

The first experiment was undertaken to determine the ability of Azotobacter to fix atmospheric nitrogen when cultivated in a definitely controlled gaseous environment. Bonazzi (4) was bold enough to suggest that this genus may not fix any free atmospheric nitrogen at all, but that it merely accumulates available combined nitrogen which may be present in the air as ammonia or nitrous acid. It is reasonable to suppose that a considerable quantity of these substances, considered from the standpoint of microörganic needs, may be present in the atmosphere under certain conditions. This would be especially true of a soil where organic decomposition is taking place. Although the amount of either ammonia or nitrous acid available at any one moment is probably exceedingly small, over an incubation period of 21 days, or more, Azotobacter may conceivably come in contact with sufficient nitrogen in these forms to account for the quantity found in the cultures.

The literature contains little evidence of any serious consideration having been given to the possibility that the nitrogen accumulation in cultures of Azotobacter may be due to the assimilation of combined rather than free atmospheric nitrogen. Winogradsky (35) in the isolation and study of *Clostridium pasteurianum*, aerated his cultures with air which had been passed through sulfuric acid. Beijerinck (2) comments on this practice, but apparently it has not been followed generally.

In order to investigate the ability of Azotobacter to fix free atmospheric nitrogen in the absence of any combined nitrogen in the air and in a medium free from combined nitrogen, the following procedure was adopted:

A series of cultures of the organism in Ashby's medium was incubated in sealed containers which were aerated daily with an atmosphere free from combined nitrogen.

A second series of cultures was set up in all respects like the first, but incubated in contact with ordinary room air which was supplied to the sealed containers in the same manner as the washed air in the first series.

Aeration was accomplished as follows: A 3-gallon bottle was filled with water and the water gradually replaced with air which had been drawn through a scrubbing tower filled with 10 per cent sulfuric acid to remove the ammonia, and through 25 per cent sodium hydroxide for the removal of nitrous acid that may have been present. The water was drawn from the bottle by a

siphon, the washed air being allowed to enter through a short intake tube; or by inverting the bottle and allowing the water to run out through a short tube in the rubber stopper, while the washed air entered through a long tube which extended from the stopper to above the water level.

After the 3-gallon bottle had been filled with washed air it was connected with the sealed container by means of rubber tubing, and the air from it was forced through the container by means of water siphoned into the first bottle from a second bottle of the same size.

Two plans of aeration were tried. The first was gradual and continous aeration for the period of incubation, fresh bottles of washed air being supplied once each day. This method was effective but required a complicated apparatus with a number of aerating bottles in use at the same time. The second plan was to aerate the containers more rapidly, the air being forced through once each day for a period of 20 minutes. This made it possible to aerate the containers outside of the incubator, and allowed the incubation of many more cultures at the same time than would have been possible with the first plan.

TABLE 1

Ability of Azotobacter to fix free nitrogen in an atmosphere from which all combined nitrogen has been removed

ORGANISM	NITROGEN FIXED PER 100 CC.	
	Ordinary room air	Air free from combined nitrogen
	mgm.	*mgm.*
A1	6.6	7.0
A3	6.4	6.4
A4	5.4	5.6
Am	8.8	8.4

The second method proved equally satisfactory and was adopted, since it allowed a more rapid accumulation of data.

It was observed that the sodium hydroxide removed the carbon dioxide from the air, and that the growth and activity of Azotobacter appeared to be greatly inhibited thereby. To counteract this influence, approximately 1 per cent by volume of carbon dioxide was added to the washed air before it was introduced into the culture containers. The influence of carbon dioxide on bacteria has been discussed by Valley and Rettger (32).

The air driven from the sealed containers during aeration was bubbled through 0.01N sulfuric acid in order to collect any ammonia which may have been given off from the cultures during incubation. The acid was titrated back with 0.01N sodium hydroxide, Congo Red being used as the indicator.

Two sets of cultures of Azotobacter were set up according to the plan outlined in the foregoing. The cultures were seeded on Ashby's agar in Blake culture bottles and incubated for three days at 28°C., after which 50 cc. of Ashby's solution was added and the cultures were incubated for 21 days at

28°C. Total nitrogen determinations were made at the end of the incubation period. Table 1 shows that the four Azotobacter strains used here are capable of fixing appreciable quantities of free atmospheric nitrogen in the absence of any combined nitrogen in the substrate and the surrounding atmosphere. Differences observed between the quantities of nitrogen fixed by the same Azotobacter strain in washed air as compared with ordinary room air are within the range of normal variation, which might be expected among cultures of the organism set up and incubated under identical conditions.

Since the foregoing experiment demonstrated the ability of Azotobacter to fix free atmospheric nitrogen in the absence of any combined nitrogen in the air or substrate, it was decided to incubate all cultures in the subsequent experiments in sealed containers which were aerated once each day with washed air.

Is Azotobacter proteolytic?

In order to test the proteolytic power of Azotobacter, inoculations of the four strains of the organism were made on Loeffler's blood serum. These were incubated for one week at 28°C. and examined. A good growth was observed in all cases, but there were no signs of liquefaction of the medium. These cultures were continued in incubation and examined from time to time until the medium became so dry that further observations were useless. The total incubation time was 40 days. At no time was there evidence of any digestion of the blood serum.

In another test the Ashby solution was employed as the basic medium and 300 gm. of gelatin to each liter added. The medium was used in tubes (slanted) and in plates. Tubes and plates were inoculated with Azotobacter and incubated at 22°C. for 20 days, after which they became too dry for satisfactory observation. Daily examination showed good growth of the organism after the third day, but there was no liquefaction of the gelatin at any time.

These tests indicate that Azotobacter is not proteolytic. The gelatin test should be especially conclusive, since inability to attack gelatin implies the lack of proteolytic power in the organism.

Influence of combined nitrogen in the substrate on growth and nitrogen fixation by Azotobacter

The chemical substances chosen for the study of the influence of combined nitrogen in the substrate on growth and nitrogen fixation by Azotobacter were as follows:

Peptone, nucleic acid, tryptophane, tyrosine, glutamic acid, aspartic acid, cysteine hydrochloride, cystine, glycocoll, creatine, creatinine, urea, guanine, ammonium sulfate, ammonium chloride, ammonium carbonate, ammonium phosphate, ammonium nitrate, sodium nitrate, and potassium nitrate.

It is obviously impossible in an investigation of this scope to determine the influence of all of the known nitrogenous compounds which may be found in soil. The compounds chosen, however, should furnish considerable evidence regarding the behavior of Azotobacter in the presence of combined nitrogen. Both simple and complex organic compounds were employed as well as some readily available inorganic substances for comparison.

The cultures were set up as indicated in the description of methods, and incubated for three days at 28°C. Then 50 cc. of Ashby's solution containing

TABLE 2

Amount of nitrogen fixed by Azotobacter in the presence of different nitrogenous compounds, and of nitrogen fixation by the same organisms in a medium having no combined nitrogen

Results are expressed as mgm. of nitrogen in 100 cc.

MEDIUM	STRAINS OF AZOTOBACTER			
	A1	A3	A4	Am
No combined nitrogen	7.0	6.4	5.6	8.4
Bacto-peptone	3.2	3.8	3.4	2.0
Nucleic acid	4.8	5.0	2.6	5.0
Tryptophane	3.1	5.2	2.4	6.0
Tyrosine	5.0	5.2	5.6	6.0
Glutamic acid	1.6	2.0	1.0	2.0
Aspartic acid	−1.2*	1.7	0.4	−0.2
Cysteine hydrochloride	1.0	0.8	0.4	0.8
Cystine	5.0	6.2	7.0	5.6
Glycocoll	3.0	2.6	3.2	2.6
Creatine	−1.8	−0.6	−0.8	−1.0
Creatinine	−2.4	−0.8	−2.6	−1.0
Urea	−2.5	2.3	−1.5	−0.7
Guanine	2.8	4.9	4.0	5.3
Ammonium sulfate	1.4	3.4	2.3	1.4
Ammonium chloride	5.1	7.8	8.2	6.3
Ammonium carbonate	6.3	7.5	5.9	3.0
Ammonium phosphate	5.0	5.5	3.8	2.6
Ammonium nitrate	6.3	8.1	6.1	1.2
Sodium nitrate	6.7	5.6	4.4	0.6
Potassium nitrate	0.0	0.6	0.0	−3.4

Indole and skatole were toxic and no growth occurred.

* The minus sign indicates that less nitrogen was recovered from the cultures after incubation than was included in the medium as combined nitrogen.

enough of the nitrogen compound employed to give the desired concentration of nitrogen was added and the cultures were incubated for 21 days at 28°C. Because of the number of compounds employed and the necessity of repeating the experiments with each several times, a standard concentration of 100 p.p.m. of nitrogen was adopted. This amount of nitrogen greatly exceeds that found in the average soil combined in any single compound.

After incubation the cultures were examined as follows:

Visual growth was observed; tests were made for ammonia and amino acids in the cultures, and for indole where the nature of the culture solution suggested their possible presence: tests were conducted for ammonia lost during incubation; finally, total nitrogen determinations were made, and the nitrogen fixed was calculated by subtracting the nitrogen content of the control cultures from that of the test cultures.

The observation of the amount of growth in the cultures may show that the increase of Azotobacter growth is greatly in excess of the increase of nitrogen

TABLE 3

Influence of combined nitrogen on the growth of Azotobacter in a fluid medium

MEDIUM	STRAINS OF AZOTOBACTER			
	A1	A3	A4	Am
No combined nitrogen	2x*	2x	2x	2x
Bacto-peptone	4x	4x	3x	4x
Nucleic acid	2x	2x	2x	2x
Tryptophane	2x	2x	2x	2x
Tyrosine	2x	2x	2x	2x
Glutamic acid	2x	2x	2x	2x
Aspartic acid	3x	3x	3x	3x
Cysteine hydrochloride	3x	3x	3x	3x
Cystine	2x	2x	2x	2x
Glycocoll	3x	3x	3x	3x
Creatine	3x	3x	3x	3x
Creatinine	3x	3x	3x	3x
Urea	4x	4x	2x	4x
Guanine	2x	2x	2x	2x
Ammonium sulfate	3x	3x	3x	4x
Ammonium chloride	4x	3x	3x	3x
Ammonium carbonate	4x	3x	3x	4x
Ammonium phosphate	4x	3x	3x	4x
Ammonium nitrate	4x	4x	3x	4x
Sodium nitrate	3x	4x	2x	4x
Potassium nitrate	3x	3x	3x	3x
Indole	00	00	00	00
Skatole	00	00	00	00

* The normal growth of each strain in the medium containing no combined nitrogen is reported in table as 2x.

fixed, or that growth has increased while nitrogen fixation is actually inhibited. The production of detectable ammonia from the nitrogenous compounds may indicate the ability of Azotobacter to attack the nitrogen compound employed in a given culture. If ammonia is liberated slowly, it may be utilized by the organism, whereas, if it is formed rapidly, it may escape from the alkaline solution during incubation. In that case, the test for ammonia lost during incubation would be useful. In cultures containing amino acids as such, in-

cluding peptone, the amino acid test should determine the amount of the amino acid remaining unused and thus indicate the ability of Azotobacter to

TABLE 4

Amount of ammonia nitrogen detected in fluid cultures of Azotobacter in the presence of combined nitrogen

MEDIUM	STRAINS OF AZOTOBACTER			
	A1	A3	A4	Am
No combined nitrogen	00	00	00	00
Bacto-peptone	1.4	3.6	2.8	1.2
Nucleic acid	Trace	Trace	Trace	Trace
Tryptophane	Trace	Trace	Trace	Trace
Tyrosine	Trace	Trace	Trace	Trace
Glutamic acid	Trace	Trace	Trace	Trace
Aspartic acid	Trace	Trace	Trace	Trace
Cysteine hydrochloride	4.2	1.4	2.1	5.6
Cystine	1.4	1.4	0.0	2.1
Glycocoll	2.1	1.4	2.1	2.8
Creatine	Trace	Trace	Trace	Trace
Creatinine	2.1	2.1	1.4	2.1
Urea	−1.7*	−0.4	−1.1	−0.4
Guanine	Trace	Trace	Trace	Trace
Ammonium sulfate	−0.8	−1.2	−0.4	−1.5
Ammonium chloride	−1.7	−1.2	−1.4	−1.4
Ammonium carbonate	0.0	−0.4	0.0	−0.4
Ammonium phosphate	−0.5	−0.5	−0.5	−0.7
Ammonium nitrate	−1.5	−0.5	−0.5	−0.8
Sodium nitrate	0.0	Trace	Trace	0.0
Potassium nitrate	0.0	0.0	0.0	0.0
Indole	0.0	0.0	0.0	0.0
Skatole	0.0	0.0	0.0	0.0

* The minus sign indicates that less ammonia was detected in the test cultures than in the control cultures.

TABLE 5

Amount of amino acid nitrogen remaining in fluid cultures of Azotobacter after 21 days' incubation

ASHBY'S MEDIUM, PLUS	AMINO ACID NITROGEN
Bacto-peptone	Trace
Nucleic acid	None
Tryptophane	One-half remained
Tyrosine	Practically all remained
Glutamic acid	One-third remained
Aspartic acid	None remained
Glycocoll	Trace

* The table represents only those cultures which had an amino acid or peptone included in the culture solution, since no amino acid nitrogen was detected in any of the other cultures of the series.

utilize amino acids. The total determination of fixed nitrogen is the main object of the investigation and needs no further explanation.

The results of the various tests are shown in the tables 2 to 6. Nitrogen fixation, which is the main objective of the experiments, is shown in table 2, growth in table 3, ammonia detected in cultures in table 4, amino acids in table 5, and ammonia lost during incubation in table 6. No indole was detected in any of the cultures.

TABLE 6

Amount of ammonia lost from fluid cultures of Azotobacter during incubation

MEDIUM	MGM. OF AMMONIA LOST PER 100 CC. OF CULTURE
No combined nitrogen	No loss demonstrated
Bacto-peptone	Average less than 0.1 mgm.
Nucleic acid	No loss demonstrated
Tryptophane	No loss demonstrated
Tyrosine	No loss demonstrated
Glutamic acid	No loss demonstrated
Aspartic acid	Average 0.8 mgm.
Cysteine hydrochloride	No loss demonstrated
Cystine	No loss demonstrated
Glycocoll	No loss demonstrated
Creatine	Average 3.2 mgm.
Creatinine	Average 0.4 mgm.
Urea	Average 1.7 mgm.
Guanine	No loss demonstrated
Ammonium sulfate	Average less than 0.1 mgm.
Ammonium chloride	No loss demonstrated
Ammonium carbonate	No loss demonstrated
Ammonium phosphate	Average less than 0.1 mgm.
Ammonium nitrate	Average 0.4 mgm.
Sodium nitrate	No loss demonstrated
Potassium nitrate	No loss demonstrated
Indole	No loss demonstrated
Skatole	No loss demonstrated

REVIEW OF DATA

Of the organic nitrogenous compounds employed, nucleic acid, tryptophane, and tyrosine did not exert any marked effect on the amount of nitrogen fixed by Azotobacter; nor was there any noticeable influence on the quantity of growth. The slight depression of nitrogen fixation suggests that the compounds may have been slightly utilized to furnish nitrogen for the use of the organism.

Peptone had a marked effect, resulting in an increase of growth and a definite depression of nitrogen fixation, as was shown by comparison with the nitrogen fixation by the same organism in a medium that was free from combined nitrogen. Commercial peptone contains many simple decomposition products of

protein digestion which may have been responsible for the pronounced influence on Azotobacter growth and activity.

Guanine, cystine, and glutamic acid depressed nitrogen fixation. This was especially true of glutamic acid. Growth was not influenced. There was no other evidence that any of these compounds was attacked by the organism. Cystine exercised the least effect of the three. It was probably present in very small quantities because of its limited solubility in the medium.

Aspartic acid, cysteine hydrochloride, glycocoll, creatine, creatinine, and urea all produced increased growth and caused marked depression of nitrogen fixation. With the last three there was actual loss of some of the original nitrogen of the medium.

Indole and skatole, in the concentrations employed, were decidedly toxic to Azotobacter.

Apparently all of the inorganic compounds were available for use by Azotobacter, growth in the liquid cultures being greatly stimulated by their presence. Nitrogen fixation was almost equal to that observed in media containing no combined nitrogen, with the exception of the experiments in which ammonium sulfate and potassium nitrate were employed. A marked exception to this rule was the behavior of organism Am. Nitrogen fixation by this strain was decidedly reduced by all of the inorganic nitrogenous salts except ammonium chloride. With potassium nitrate some of the original nitrogen was lost in cultures of Am. This loss was the greatest noted in any of the cultures in which combined nitrogen was employed.

A comparison of the growth of Azotobacter in the presence of inorganic nitrogenous salts with the amount of nitrogen fixed indicates that the metabolic efficiency of the organism was greatly inhibited.

Definite quantities of ammonia were detected in cultures containing peptone, cysteine hydrochloride, glycocoll, and creatinine. It seems probable that this ammonia resulted from decomposition of the compounds by Azotobacter, and that if the evolution of ammonia is slow enough, the organism will utilize it for conversion to protoplasmic nitrogen. No ammonia was observed in cultures containing creatine, in spite of other evidence that Azotobacter had utilized this substance.

The loss of ammonia from cultures containing creatine was the greatest observed among the experiments. Probably the material was attacked so vigorously that the ammonia escaped from the medium before it could be utilized. Some ammonia was also lost during incubation from cultures containing aspartic acid, creatinine, urea, and ammonium nitrate.

GENERAL DISCUSSION

The ability of Azotobacter to fix atmospheric nitrogen in the complete absence of combined nitrogen was definitely demonstrated with four different strains in the first experiment. It seems reasonable to assume that other

strains of the organism would possess the same power. Although it is improbable that laboratory air, under ordinary conditions, would contain a measurable quantity of ammonia or nitrous acid at any one moment, the exposure of the cultures to minute amounts of these substances over a 3-week incubation period may have a decided influence on the behavior of the organisms. The present experiments were carried out, therefore, in an atmosphere free from combined nitrogen.

In considering the influence of combined nitrogen in the medium on growth and nitrogen fixation by Azotobacter, the results obtained do not agree entirely with those reported by Hills (13), who found that nitrogen fixation was stimulated by the presence of ammonium, sodium, and potassium nitrates. No such stimulus was observed in this investigation. Hills' experiments were carried out in soil which naturally may be expected to contain some nitrates, with a consequent complication of results. Another factor in his work which seems worthy of consideration is the interpretation of the relation of the quantity of growth to nitrogen fixation. He reported that growth was stimulated by the presence of nitrates. If the total-nitrogen figure is considered alone, it can be said that nitrogen fixation was stimulated. If the efficiency of the individual organism is considered, then it is logical to conclude that the nitrogen fixation by each individual cell was inhibited.

Reed and Williams (25) found that urea, glycocoll, and creatinine were only slightly depressive in their effect on nitrogen fixation. In the present investigation creatinine and creatine were both readily utilized and the nitrogen-fixing function entirely suspended. Reed and Williams do not appear to offer any evidence, other than the effect on nitrogen fixation, to indicate that Azotobacter is able to attack these substances.

Zoond (37) stated that minute traces of potassium nitrate, tyrosine, and Bacto-peptone depressed nitrogen fixation by Azotobacter cultures. The present findings do not agree in the instance of tyrosine, which had little or no effect.

The possible influence of some other factor than nitrogen, such as the sulfur of ammonium sulfate, cysteine, and cystine, must be considered in the interpretation of results. Greaves (8) states that azofiers require sulfur for the formation of the protein material of their bodies.

Bonazzi (4) states that it is a common experience to observe an increase of Aztobacter growth in the presence of nitrates. On the other hand, Waksman (33) suggests that among the reasons for the decreased fixation of nitrogen by Azotobacter in the presence of nitrates is the toxic effect which the nitrate salts exert on Azotobacter growth. The results obtained in this investigation indicate that nitrates are not toxic in quantities likely to be encountered in normal soil. Hills' (13) work agrees with this statement.

It was observed in these experiments that indole and skatole are toxic to Azotobacter in the concentrations used. Reed and Williams (25) report the same result with skatole. They do not report any experiments with indole.

The fact that these compounds are toxic for Azotobacter in relatively small quantities suggests the possibility that they may exercise the same effect in soil at times, as they are likely to be present as decomposition products of protein.

A survey of the findings of the foregoing experiments suggests that Azotobacter will utilize available combined nitrogen where possible instead of following the more laborious process of fixing free nitrogen. One is reminded of the opinion expressed by Bonazzi (4), that Azotobacter has the power to fix free atmospheric nitrogen, but that the function is only operative in case of necessity, and not always, or even often, for the benefit of man.

Table 2 shows that the behavior of strain Am in the presence of ammonium carbonate, ammonium phosphate, ammonium nitrate, sodium nitrate, and potassium nitrate is very different from that of the other Azotobacter strains employed. This fact suggests that care should be used in making general assertions regarding the behavior of Azotobacter from data obtained with a few strains.

SUMMARY

Azotobacter appears to be able to fix substantial quantities of free atmospheric nitrogen when cultivated in a medium which is free from combined nitrogen and in an atmosphere which is free from ammonia and nitrous acid.

Azotobacter did not attack blood serum or gelatin.

Nucleic acid, tryptophane, and tyrosine depressed nitrogen fixation slightly, and did not influence the growth of Azotobacter.

Peptone increased the growth of the organism, but decreased nitrogen fixation.

Glutamic acid, aspartic acid, cysteine hydrochloride, and glycocoll appeared to be readily utilized by Azotobacter. Nitrogen fixation was definitely depressed, however, and in some cases some of the original nitrogen was lost. Growth was increased in all cultures.

Cystine had little effect, probably because of its limited solubility in the medium.

Creatine, creatinine, and urea caused increased growth in most of the cultures, but depressed nitrogen fixation almost entirely, In some of the cultures there was loss of some of the original nitrogen. Guanine caused depression of nitrogen fixation, but did not influence growth.

Indole and skatole were toxic in concentrations as low as 50 p.p.m. of nitrogen equivalent.

Ammonium sulfate and potassium nitrate depressed nitrogen fixation and at the same time increased growth.

Ammonium chloride, ammonium carbonate, ammonium phosphate, amonium nitrate, and sodium nitrate produced a greatly increased growth in a majority of the cultures, and only a very slight depression of nitrogen fixation,

except in some of the cultures of strain Am. When the amount of growth is correlated with nitrogen fixation, a relative depression of fixation is apparent.

The following general observations seem to be justified:

The more complex organic compounds, except peptone, do not seem to be actively attacked by Azotobacter, and consequently the growth and nitrogen-fixing activity of the organism are not much influenced. The presence of simple nitrogenous compounds in commercial peptone is in all probability responsible for the results observed.

The simpler organic substances, including the lower amino acids, and the inorganic compounds seem to be utilized easily by Azotobacter, with the result that growth is increased and nitrogen fixation either relatively or actually depressed.

Where there is adequate evidence that the combined nitrogen is available for Azotobacter, it appears to be used in preference to the more laborious process of fixing free atmospheric nitrogen.

REFERENCES

(1) Ashby, S. F. 1907 Some observations on the assimilation of atmospheric nitrogen by a free-living soil organism, *Azotobacter chroococcum* of Beijerinck. *Jour. Agr. Sci.* 2: 35–51.

(2) Beijerinck, M. W. 1901 Ueber oligonitrophile Mikroben. *Centbl Bakt.* (II) 7: 561–582.

(3) Beijerinck, M. W., and Van Delden, A. 1902 Ueber die Assimilation des freien Stickstoffs durch Bakterien. *Centbl Bakt.* (II) 9: 3–43.

(4) Bonazzi, A. 1921 Studies in *Azotobacter chroococcum*, Beijerinck. *Jour. Bact.* 6: 331–369.

(5) Brown, J. H. 1923 The formol titration of bacteriological media. *Jour. Bact.* 8: 245–264.

(6) Conn, H. J. 1918 The microscopic study of bacteria and fungi in the soil. N. Y. (Geneva) Agr. Exp. Sta. Bul. 64.

(7) Dojerinko 1902 Cited from Jodidi, (17).

(8) Greaves, J. E. 1918 Azofication. *Soil Sci.* 6: 163–217.

(9) Greaves, J. E., and Carter, E. G. 1916 Influence of barnyard manure and water upon the bacterial activities of the soil. *Jour. Agr. Res.* 6: 889–926.

(10) Greaves, J. E., and Nelson, D. H. 1923 The influence of nitrogen in the soil on azoification. Utah Agr. Exp. Sta. Bul. 185.

(11) Headden, W. P. 1912 Deterioration in the quality of sugar beets due to the nitrates formed in the soil. Colo. Agr. Exp. Sta. Bul. 183.

(12) Heinze, B. 1910 Bodenbakteriologische Untersuchungen. *Landw. Jarhb.* 39: 515–543.

(13) Hills, T. L. 1918 Influence of nitrates on nitrogen-assimilating bacteria. *Jour. Agr. Res.* 12: 183–230.

(14) Jodidi, S. L. 1910 Organic nitrogenous compounds in peat soils. *Jour. Amer. Chem. Soc.* 32: 396–410.

(15) Jodidi, S. L. 1911 Chemical nature of the organic nitrogen in soil. *Iowa Agr. Exp. Sta. Res. Bul.* 3: 113–154.

(16) Jodidi, S. L. 1912 The behavior of amino acids in the soil. *Orig. Com., VIII, Internatl. Cong. Appl. Chem.* 26: 119–134.

(17) Jodidi, S. L. 1913 The chemistry of soil nitrogen. *Jour. Franklin Inst.* 175: 483–488.

(18) Krainski, A. V. 1912 Enriching of soil in nitrogen in connection with the life activity of aerobic microorganisms assimilating free nitrogen. Cited from Greaves (8).

(19) Lipman, J. G. 1904 Experiments on the transformation and fixation of nitrogen by bacteria. *N. J. Agr. Exp. Sta. Ann. Rpt.* 903: 217–285.

(20) Löhnis, F., and Green, H. H. 1914 Ueber die Entstehung und die Zersetzung von Humus, sowie ueber dessen Einwirkung auf die Stickstoff Assimilation. Cent. f. Bakt., Abt. II, 40, 52–69.

(21) McBeth, I. G. 1913 Cellulose as a source of energy for nitrogen fixation. *U. S. Dept. Agr., Bur. Plant. Indus. Cir.* 131: 25–34.

(22) McFarland, J. 1907 The nephelometer. *Jour. Amer. Med. Assoc.* 48: 1176–1178.

(23) Mockeridge, F. A. 1912 Some conditions influencing the growth of Azotobacter and the fixation of nitrogen by the organism. *Ann. Bot.* 26: 871–887.

(24) Neidig, R. E., and Snyder, R. S. 1926 The relation of the yield and protein content of wheat to the nitrogen content of the soil under ten years of different systems of cropping. Idaho Agr. Exp. Sta. Res. Bul. 5.

(25) Reed, H. S., and Williams, B. 1915 The effect of some organic soil constituents upon nitrogen fixation by Azotobacter. *Va. Agr. Exp. Sta. Tech. Bul.* 4: 81–95.

(26) Richards, E. H. 1917 The fixation of nitrogen in feces. *Jour. Agr. Sci.* 8: 299–311.

(27) Russell, E. J. 1927 Soil Conditions and Plant Growth, ed. 5, 178 and 247. Longmans, Green and Co.

(28) Schreiner, O., and Skinner, J. J. 1912 Chemical nature of soil organic matter. U. S. Dept Agr., Bur. Soils Bul. 87.

(29) Sestini 1898 Cited from Jodidi (17).

(30) Stoklasa, J. 1908 Beitrag zur Kenntnis der chemischen Vorgänge bei der Assimilation des elementaren Stickstoffs durch Azotobacter und Radiobacter. *Cent. Bakt.* (II) 21: 484–509, 620–632.

(31) Stranak, Fr. 1909 Zur Assimilation des Luftstickstoffs durch im Boden freilebende Mikroorganismen. *Cent. Bakt.* (II) 25: 320.

(32) Valley, G., and Rettger, L. F. 1927 The influence of carbon dioxide on bacteria. *Jour. Bact.* 14: 101–137.

(33) Waksman, S. A. 1927 Principles of Soil Microbiology, p. 575. Williams and Wilkins, Baltimore, Md.

(34) Warrington 1887 Cited from Jodidi (17).

(35) Winogradsky, S. 1895 Rechérches sur l'assimilation de l'azote libre de l'atmosphere par les microbes. *Arch. Sci. Biol. (St. Petersburg)* 3: 297–352.

(36) Winslow, C.-E. A., and Falk, I. S. 1926 A contribution to the dynamics of toxicity and the theory of disinfection. *Jour. Bact.* 11: 1–25.

(37) Zoond, A. 1926 The relation of combined nitrogen to the physiological activity of Azotobacter. *Brit. Jour. Exp. Biol.* 4: 105–113.

A LARGE SAND CULTURE APPARATUS

FRANK M. EATON

Bureau of Plant Industry, U. S. Department of Agriculture

Received for publication May 21, 1930

A large sand culture apparatus designed to permit the growing of comprehensive groups of crop plants to mature stages under comparable conditions is here described. Empirical proofs showing that plants differ greatly in their nutritional requirements, tolerances, and absorption rates, indicate a need for a suitable method for obtaining comparative information of this kind for the more important plants and the essential and toxic elements. The apparatus here described has been used for the past year at this laboratory in a study of the growth reactions of crop plants to different concentrations of boron. The equipment has been found satisfactory for the purpose indicated and it seems probable that it may be applicable to other nutritional studies as well.

In the boron work, outstanding differences have been found not only in the requirements and tolerances of the plants compared but also in the amounts of boron which the different plants absorbed. Information so gained has proved to be of direct value in itself and it is providing a basis for the interpretation of field observations. A knowledge of the relative behaviours of the different plants is likewise helpful in the selection of test plants best suited for critical studies of significant physiological differences and of factors which influence boron tolerance.

Many of the sand culture methods which have been described in the past require an apparatus which is not only elaborate in construction but which is exacting in the amount of attention and time necessary for its maintenance and manipulation. None of the methods with which the writer was familiar avoided the difficulties encountered in the use of a large number of individual pots. If individual pots are used for each of the different plants compared it is practically certain that the concentration of the ion of interest will vary around a different mean in each of them. These inequalities can be reduced by the use of large volumes of culture solution but the replacements of the culture solutions necessarily must be frequent since plants of different size and kind have different transpiration and absorption rates. The sand culture beds shown in figure 1 and plate 1 provide a rapid and simple method for the daily or more frequent replacement of culture solutions, and in the large sand beds space is available for extensive spreading and overlapping of roots. This overlapping of roots in the culture medium, which is common to all of the plants

receiving the same treatment, serves to equalize the concentration of the solution available to the different plants between the successive replacements.

Information of the general type indicated may be obtained in some cases from differently treated field plots, but under field conditions there are numerous uncontrolled factors, and uncertainties are introduced by soil heterogeneity, chemical reactions in the soil, and by the vertical distribution of the element in question in relation to the root systems of the different plants. Soils with initial characteristics which cause them to be at once suitable for studies on the effects of both a minimum and a large amount of a particular element are usually difficult to find.

SAND CULTURE APPARATUS

A set of five sand beds is shown in plate 1 as they have been set up and used at this laboratory. A line sketch illustrating one of the beds with its solution barrels and plumbing is shown as figure 1.

In operation the procedures are as follows: A cloth bag is suspended through the opening of the lower of the two barrels and into this bag are placed the weighed portions of the different salts required for the nutrient solution. Water is then allowed to flow from a hose through this bag until the salts are dissolved and the barrel is filled. It is desirable to add the readily soluble calcium nitrate to the bags after the other salts have been dissolved. With the valves at *A* and *B* closed the contents of the lower barrels are drawn into the upper barrels by exhausting the air from the latter by means of an air line connected to a filter pump on a faucet at one of the laboratory sinks. This air line is common to each of the five barrels and no valves are used. About one hour is required for lifting the solutions from the lower to the upper barrels. To flood the sand beds the valves at *B* and *C* are closed and those at *A* are opened. The whole of the solution in the upper barrel can be passed through the sand by opening the valves at *C* slightly as soon as the culture solution has reached the surface of the sand. The by-pass, with valve *B*, is necessary only when the beds are flooded from below, which should be done occasionally to prevent the sand from becoming compacted in the beds.

The frequency with which the culture solutions are discarded, by allowing them to escape through valve *D*, is dependent upon the character of the experiments. As the boron experiments are conducted the beds are flooded daily with the same solution for from 12 to 8 days during the seedling stages but as the plants become larger and their transpiration rates increase, new solutions are substituted for the old as often as every five days. It has been found that the original boron concentrations can be maintained in the solutions for a week or longer by adding sufficient water to replace three-quarters of the daily transpiration. The rate of boron absorption by plants is dependent, however, upon a number of factors and though good growth can be maintained for considerable periods by replenishing the solution with water it has not been considered

desirable in the boron experiments to add more than a total of 50 gallons of water to any of the solutions before they are discarded.

Daily conductivity measurements made on samples collected from the lower barrels provide a convenient method for following the changes in concentrations which take place in the culture solutions with use. Or, if an easily determinable ion is of primary interest, a daily analysis will indicate the amount of water, or of the element, which should be added to a solution to bring it

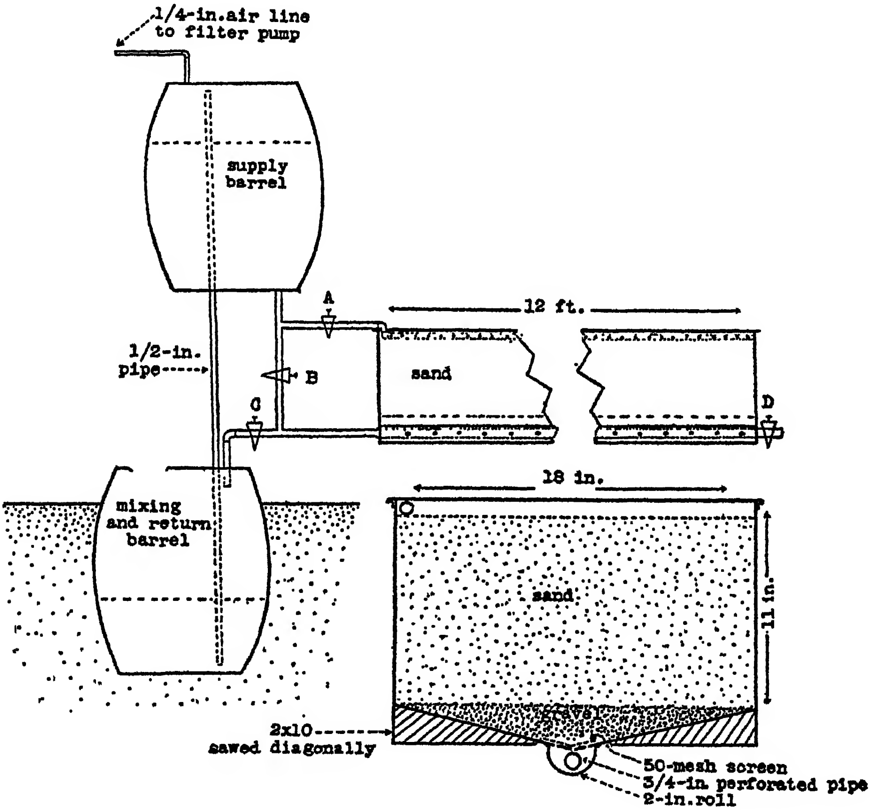


FIG. 1. SECTION OF SAND CULTURE APPARATUS, SHOWING SANDBED, SOLUTION BARRELS, AND PLUMBING

back to its original strength. Although it has not been tested, it appears probable that a sample collected from valve *D* before *C* is opened should be closely representative of the solution displaced from the sand after a day's use.

With beds of the dimensions indicated, approximately 25 gallons of nutrient solution is required to flood the sand in a bed after it has been once brought to saturation and drained. Starting with an air-dried sand, a full barrel, 50 gallons, is needed for the first flooding.

A culture solution which has been used and found satisfactory for the growth and maturity of most of the crops tested is as follows:

$Ca(NO_3)_2 \cdot 4H_2O$	6 millimoles per liter,	268.1 gm. per 50 gallons
$MgSO_4 \cdot 7H_2O$	3 millimoles per liter,	139.9 gm. per 50 gallons
KH_2PO_4	3 millimoles per liter,	77.3 gm. per 50 gallons
Mn as $MnCl_2$	0.5 p.p.m.,	0.22 gm. per 50 gallons
B as H_3BO_3	1 p.p.m.,	1.08 gm. per 50 gallons

In addition, 20 cc. of a 5 per cent solution of iron tartrate is added to each barrel each time the beds are flooded.

The details of size and arrangement of the apparatus can, of course, be varied to meet individual needs. The tanks as used in this laboratory were constructed in a sheet metal shop on the following specifications:

Five 20-guage, rust resistant tanks.—These tanks are to be 12 feet long and 18 inches wide. The depth at the sides is to be 11 inches and at the center line 12½ inches. The top of each tank is to be reinforced with 1-inch angle iron which is to be riveted in place and covered by bent-over extensions of the galvanized iron of the tanks. A 1-inch angle iron tie rod is to be placed across the tops of the tanks and riveted to the side angle irons at 4 feet from each end of the tanks. Along the center line of the bottoms of the tanks there are to be 3 rows of ⅛-inch holes. These rows are to be ½-inch apart and the holes in the rows 2 inches apart.

Roll on bottom of each tank.—A 2-inch galvanized iron roll is to be soldered and riveted to the bottom of each tank. *Galvanized pipes in rolls.*—A ¾-inch galvanized pipe with a ¼-inch hole drilled through at each 8 inches is to be supplied and secured in each roll as follows: These pipes, 148 inches long, are to have a 2 inch running thread at each end which will extend through holes in a 16-guage plate which has been riveted and soldered to the ends of the rolls and tanks. Soldered to the plate at one end of the tank there is to be a 1 inch waste nut. A 1 inch by ¾-inch bushing is to be screwed over the end of the pipe and into this waste nut. The pipe at the other end of the tank will be secured in place with a gasket and lock-nut, leaving 2 inches of projecting pipe at each end. Before each pipe is finally secured in place, the holes in the end plates are to be closed with stoppers and a quart of asphaltum paint pored into the roll. The tanks are then to be tipped and rolled until all exposed metal within the rolls is covered. The galvanized pipe and the inside of the tank and the outside seams are to be given a good coat of asphaltum paint.

Before the beds were filled with sand a strip of asphaltum painted 50-mesh copper screen was laid over the holes in the bottom of the tanks and on top of this was placed 2 inches of pea gravel. Clean quartz sand, sufficiently fine for all but a small fraction to pass a 40-mesh screen, was used to fill the beds. The screen and gravel used in the bottoms of the tanks effectively prevent sand from passing into the rolls or drain pipes. The ½ inch galvanized pipe at the surface of the sand is provided with an ⅛ inch hole at each 6 inches. These holes are set at an angle of 45° with the sand.

The cost of each of the units including the tank, two 50-gallon barrels, plumbing, plumbing supplies, supports, and labor of setting up, was $75.00, of which amount the cost of each of the tanks constructed according to the above specifications was $35.00. The beds were set on frames constructed of 2 inch by 6 inch lumber, supported by hollow tile as shown in plate 1. The barrels

were mounted on a frame constructed of 2 inch by 12 inch lumber similarly supported with hollow tile.

The apparatus described was designed for experimental use in the open but it appears that it might be well suited for purposes of propogation in greenhouses in place of soil benches, particularly when a ready means of soil sterilization by steam or chemicals is essential. For such a purpose the supply barrels could be located in a corner of the headhouse and if the water was regularly added to replace that lost by transpiration, only an occasional renewal of the entire culture solution would be necessary. Under such conditions the size of the sand beds, relative to the capacity of the supply barrels, could be increased.

PLATE 1

Sand Beds as Set Up and Used at the Rubidoux Laboratory, Riverside, Calif.

The culture solution used in the bed in the foreground contained 25 p.p.m. of boron. This quantity of boron was sufficient to kill a number of the plants tested and to reduce greatly the growth of many.

LARGE SAND CULTURE APPARATUS

FRANK M. EATON

BOOK REVIEWS

Tropical Soil Forming Processes and the Development of Tropical Soils—With Special Reference to Java and Sumatra. By E. C. J. MOHR.

This was originally published in Dutch, but has been translated to the English by Dr. Robert L. Pendleton, professor of soil technology, College of Agriculture, University of the Philippines, and published in mimeographed form as Experiment Station Contribution No. 655. It consists of 213 pages including an excellent index. The first 52 pages discuss processes of soil formation under tropical conditions. The rest of the publication describes the character of the soils together with a discussion of their formation throughout Java and Sumatra.

CHARLES F. SHAW.

Principles of Agrobiology, or the Laws of Plant Growth in Relation to Crop Production. By OSWIN W. WILLCOX, Ph.D. Palmer Publishing Corp., Inc., 153 Waverly Place, New York, 1930. Cloth, 96 pp., double column, figs. 54.

In this book, designed as a text for practicing agronomists and teachers and students of crop nutrition, the author erects agrobiology as an essentially new science covering "that division of agricultural science which considers the general external relations of crop plants to their environments and their reactions as massed colonies to the action of growth factors." The laws referred to in the subtitle are the laws of: constancy of types; definite growth powers; universality of essential growth factors; constancy of the action of growth factors; joint action of growth factors; definite optima; diminishing increments of yield; increasing increments of yield; concentration law of growth factors; logistic law of crop growth; action law of growth factors; effect law of growth factors; homologous yield curves; conflicting attributes of growth factors; the real law of the minimum; calorific duty of vegetable nitrogen; and crop-fertilizer ratios. All of these laws are objectively illustrated by examples from crop records and large-scale investigations. Concepts new to the literature of crop growth are the limits to the action of growth factors and the corresponding limits to crop growth; the differentiation of subultra and perultra crop plants; the definition of perfertile soil, perultimate yield, and satiation pressure (growth inertia); the absolute scale of the growth power of plants; the calorific duty of vegetable nitrogen; and a plant physiological explanation for the law of diminishing increments of yield. Quite enough, seemingly, to

justify the separation of agrobiology as a distinct and entirely new branch of the science of plant life. Fertilizer statics, or the maintenance of soil fertility, is treated in the light of the Mitscherlich effect law. The mathematics of the Mitscherlich and the Spillman yield equations and their practical applications are treated in an appendix.

M. Ludtke.

MIKROBIOLOGIE
TECHNISCHE HOOGESCHOOL

Martinus Willem Beijerinck
1851–1931

In a country home, close to Gorssel, in the eastern part of Holland, died, on January 1 of this year, Prof. M. W. Beijerinck, whose work in the field of microbiology in general and of soil microbiology in particular is of the greatest theoretical and practical importance.

Beijerinck's first outstanding scientific contribution was of a botanical nature, on a subject bordering between botany and microbiology, namely, his work on the plant galls, which appeared in 1877. His interest in microbiology was aroused in 1885 when he was invited, at the recommendation of Hugo de Vries, by the Netherland Yeast and Alcohol Factory to undertake a series of studies of the growth of microörganisms. This resulted in an investigation, published in 1887, on the rôle of free oxygen in the life processes of the organisms bringing about fermentation. In this work he was a direct successor of Pasteur, who 25 years previously had elucidated the principles of anaerobiosis as "life without oxygen."

It was, however, in 1888, that Beijerinck announced to the world the isolation of a bacterium concerned in the formation of nodules on the roots of leguminous plants, the now famous *Bacillus radicicola*. This was the first of a series of brilliant contributions to the subject of soil microbiology, which has helped in attracting universal attention to the rôle of microörganisms in soil processes and plant growth.

In 1895, Beijerinck was appointed professor of bacteriology at the Polytechnical School in Delft. It was in this laboratory that the most outstanding work in microbiology by Beijerinck and his pupils and associates, who came to him in increasing numbers, was carried out. The investigation of the sulfate-reducing bacteria (*Spirillum desulfuricans*) appeared in 1896, of *Azotobacter* in 1901, of the sulfur-oxidizing bacteria (*Thiobacillus* group) in 1903, of the bacteria bringing about denitrification in 1903, all of which are of the greatest importance in soil science. In addition to these, Beijerinck's investigations dealt with the butyl alcohol and butyric acid bacteria, the lactic acid and acetic acid bacteria, the microbes active in the retting of flax, the microorganisms of kefir, as well as with the nutrition of algae, amoebae, yeasts, and fungi, embracing nearly every branch of microbiology. Plant pathology benefited considerably not only from his work on the root galls, but also from his study of the contagious nature of "gummosis" (1882), as well as of the mosaic in tobacco plants ("contagium vivum fluidum").

Beijerinck will always be considered in the history of microbiology as a

pioneer who opened up numerous fields of investigation. In this respect, he is a direct descendant of his likewise great countryman Antonie van Leeuwenhoek. His work was progressing so rapidly, and numerous ideas and discoveries were made in such rapid succession that, although he made several attempts to write an all-embracing book, such as the tentative *Ecology of Microbes*, he never wrote it.

Beijerinck's investigations pointed the way to numerous applications of the activities of microörganisms in natural processes. At an age when the microbes as pathogenic organisms were attracting universal attention, the work of Beijerinck emphasized the useful functions of the numerous microbes in agriculture and in industry. Beijerinck's contributions to soil science bridged the gap between the growth of plants and soil processes, as indicated by his work on the root galls and nodule bacteria. Toward the end of his life he returned to botany, as indicated by his papers on regeneration of buds and position of leaves. Although botany and plant anatomy, microbial physiology and microbial ecology will always be indebted to him for his great contributions to the respective sciences, it is soil microbiology that has benefited most from the work of this man of genius.

His numerous pupils, his still larger number of friends and visitors who have gained from his magnetic personality, and all those who were guided in their work by his ideas and discoveries will deplore deeply the loss that science suffers from the passing of this indefatigable investigator.

SELMAN A. WAKSMAN.

SOIL ORGANIC MATTER-TEMPERATURE RELATIONSHIP IN THE EASTERN UNITED STATES

HANS JENNY

University of Missouri

Received for publication June 7, 1930

In the Great Plains region and in the prairie-timber transition zone the average total nitrogen content of the soil decreases with increasing annual temperature in regions of similar precipitation-evaporation ratios (1, 3).

Since the total nitrogen and the total organic carbon content of the soil form a more or less constant ratio, as demonstrated by Sievers and Holtz, Waksman, and others (5), the organic matter content of the soil should manifest a similar decrease from north to south in relation to temperature.

This paper is a report of a study to determine whether a soil organic matter-temperature relationship can be observed quantitatively. The region selected for this investigation belongs to the eastern United States, including New York, New England, New Jersey, Delaware, Maryland, Virginia, North Carolina, South Carolina, Georgia, Alabama, and Florida. The parent material from which the soils are derived consists partly of igneous and metamorphic rocks, (Piedmont Plateau) and partly of sedimentary deposits (Coastal Plains). The native vegetation has been classified by Shantz as "southern hardwood forest" (oak-pine phase) and "southeastern pine forest" (longleaf-loblolly-slash pines).

Organic matter analyses (total carbon x 1.742) of 18 counties, lying mainly within the Piedmont Plateau and the Atlantic Coastal Plain, were taken from the *Field Operations of the Bureau of Soils 1902 and 1903*. Only predominating, well-drained upland soils of loam and sandy loam textures were considered. Each point in figure 1 represents the average organic matter content of the surface soils of an entire county, which was calculated according to the example given in table 1.

In order to follow the trend of the organic matter-temperature relation north of the Piedmont Plateau and Atlantic Coastal Plain, three counties from New York and New England were included in this investigation. The humidity factors of these counties are somewhat higher, namely N. S. Q.[1] 400–500, than those of the southern counties, which lie between N. S. Q. 300–400. Consequently in fitting the curve, less weight was given to the three northern points in the graph, which would make the slope of the curve even steeper than that shown in figure 1 (see also table 2).

[1] Rainfall divided by absolute saturation deficit of air (2).

The correlation ratio, which is considered to be an index of the dispersion of the points around the curve, has a value of -0.87 ± 0.036. According to the scale of Chaddock, this value indicates a high degree of association between temperature and soil organic matter.

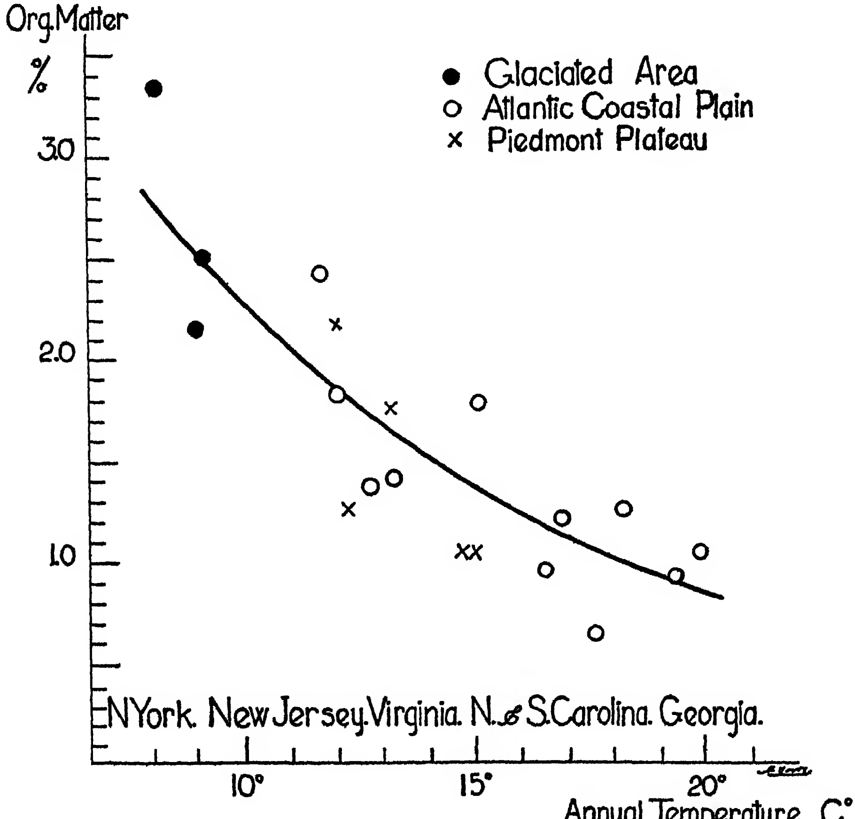


FIG. 1. ORGANIC MATTER-TEMPERATURE-DIAGRAM OF SOILS FROM THE EASTERN UNITED STATES

Each point represents a "county average"

The computation of the "calculated values" in table 2 is based on the empirical formula:

$$O.M. = 6.50\, e^{-0.104\, T_{-}^{20}}$$

where $O.$ $M.$ means average organic matter content of the surface soils of a county in per cent and T the mean annual temperature of a county in degrees C.

The rule previously observed, that for a fall of 10°C. in annual temperature, the average nitrogen content of the soil increases two to three times, is also valid for the organic matter content of the soils investigated. At 20°C. the

average calculated organic matter content is 0.81 per cent, whereas at 10°C. it reaches 2.30 per cent, which is 2.84 times greater than the lower value at 20°C.

A very interesting property of the soil organic matter-climate relationship becomes evident by comparison of the organic matter figures in table 2 with

TABLE 1

Illustration of the method of calculating the average organic matter content of the soils of a county

Perry County, Alabama. Ann. Temperature 64.5°F. Ann. Humidity factor N. S. Q. about 310.

SOIL TYPES (COSTAL PLAIN SOILS)	PER CENT OF COUNTY AREA	ANALYSES OF ORGANIC MATTER CONTENT		AREA × AVERAGE	AVERAGE ORGANIC MATTER CONTENT OF COUNTY
		Single values	Average		
		per cent	*per cent*		*per cent*
Orangeburg sandy loam	40.2	0.38, 0.70, 0.54, 0.67	0.57	40.2 × 0.57 = 22.91	$\frac{27.41}{43.2}$ = 0.63% O.M.
Sassafras sandy loam	3.0	2.01, 1.00	1.50	3.0 × 1.50 = 4.50	
	43.2			27.41	

This method of calculation might be called a mathematical "short cut" of the following reasoning: In calculating the average organic matter content of the county, not only the organic matter content of the various soil types, but also their area, has to be taken into consideration. Thus, in the above example the county organic matter content, if based solely on the analyses or the two soil types, is $\frac{0.57 + 1.50}{2} = 1.035$ per cent, which is not a fair value, because the abnormally high organic matter value of the unimportant soil type Sassafras sandy loam receives too large a share in forming the county average. This error is corrected by using the area of the soil types as "weight." In the above case, the area of Perry County is 487,744 acres. The Orangeburg sandy loam occupies 40.2 per cent, or 196,073 acres, the Sassafras sandy loam 3.0 per cent, or 14,632 acreas. The organic matter percentage multiplied by 20,000 gives the organic matter content in pounds to the acre, 6⅔ inches deep (2 million pounds of soil), which is 11,400 pounds for the Orangeburg sandy loam and 30,000 pounds for the Sassafras sandy loam. Multiplying pounds of organic matter to the acre by the number of acres gives the total amount of organic matter present in the entire area of the respective soil types. Hence, for Orangeburg sandy loam: $11{,}400 \times 196{,}073 = 2.235 \times 10^9$ pounds, and for the Sassafras sandy loam: $30{,}000 \times 14{,}632 = 0.439 \times 10^9$ pounds. The 210,705 acres (both soil types together) contain therefore 2.674×10^9 pounds of organic matter, or one acre contains 2.674×10^9 divided by $210{,}705 = 12{,}691$ pounds to the acre. Dividing this value by 20,000 converts the pound to the acre value into the percentage figure, which gives 0.63 per cent organic matter.

the nitrogen values of the timber soils from the Middle West, which were published previously (1, p. 176). The timber soils of Wisconsin, Illinois, Western Kentucky and Tennessee, and Mississippi are subject to annual climatic conditions similar to those of the Atlantic Coast States, consequently,

one might also expect a certain conformity within the soil organic matter constituents of the two regions. Several calculated nitrogen averages of midwestern timber soils are shown in column 3 of table 3; the climatically corresponding organic matter figures from the eastern soils are listed in column 4. Division of the latter values by the conventional humus factor 1.742 gives the percentages of total organic carbon (column 5). If one divides now the carbon figures of the eastern soils by the climatically corresponding nitrogen figures of the midwestern soils, one obtains a surprisingly constant

TABLE 2

Summary of organic matter values

ANNUAL TEMPERATURE		STATE	COUNTY AREA	NUMBER OF SOIL TYPES PER COUNTY	NUMBER OF ANALYSES PER COUNTY	AVERAGE ORGANIC MATTER CONTENT OF LOAMY SOILS PER COUNTY				
						Glaciated region	Piedmont region	Costal plain	Calculated value	Deviation
°F.	°C.					*per cent*	*per cent*	*per cent*	*per cent*	
46.7	8.17	New York	Syracuse	3	8	3.35	....	....	2.78	+0.57
48.2	9.00	Connecticut Valley		4	7	2.16	....	....	2.55	−0.39
48.4	9.11	New York	Big Flats	2	6	2.51	....	....	2.52	−0.01
52.1	10.67	New York	Long Island	3	7	....	....	2.43	2.03	+0.40
53.6	12.00	New Jersey	Trenton	5	14	....	2.18	1.82	1.87	+0.31 −0.05
53.8	12.17	Virginia	Leesburg	6	13	....	1.27	....	1.83	−0.56
55.0	12.78	Delaware	Dover	2	5	....	....	1.37	1.72	−0.35
55.8	13.22	Virginia	Albemarle	2	5	....	1.74	....	1.64	+0.10
56.0	13.30	Maryland	Worcester	2	6	....	....	1.42	1.63	−0.21
58.7	14.83	N. Carolina	Hickory	2	6	....	1.09	....	1.39	−0.30
59.1	15.06	S. Carolina	Campobello	1	2	....	1.09	....	1.36	−0.27
59.4	15.22	Virginia	Norfolk	3	9	....	....	1.79	1.33	+0.46
61.9	16.61	N. Carolina	Graven	2	6	....	....	0.98	1.16	−0.18
62.5	16.94	S. Carolina	Darlington	5	9	....	....	1.22	1.12	+0.10
63.9	17.72	Alabama	Perry	2	6	....	....	0.63	1.03	−0.40
64.9	18.28	Georgia	Fort Valley	2	4	....	....	1.27	0.97	+0.30
67.0	19.44	Alabama	Mobile	3	7	....	....	0.95	0.86	+0.09
68.0	20.00	Florida	Gadsden	3	8	....	....	1.06	0.81	+0.25

value of about 10.8 (column 6) which represents the well-known carbon-nitrogen ratio. In other words, the eastern organic matter-temperature curve and the midwestern nitrogen temperature curve are identical in that they differ only by a constant, the C/N ratio times 1.742. Multiplying, for example, the calculated average nitrogen content of central Illinois timber soils, which is 0.122 per cent N (at 10°C.), by 10.8 x 1.742 results in an organic matter content of 2.29 per cent. The corresponding organic matter value of the eastern soils at 10°C. is 2.30 per cent. This close agreement is undoubtedly due to the fact that all of the calculated figures are based on averages of large

regions. Taking observed data of smaller areas instead of calculated regional averages, naturally makes the agreement less close. For the particular district of Illinois in question, the analytical averages read as follows: N = 0.124 per cent; C:N ratio = 11.21, organic matter = 2.42 per cent. The difference between calculated and observed organic matter and C:N figures amounts to 5.5 per cent. In this kind of work, dealing with so many uncontrolled variables, an agreement within 10 per cent must be considered satisfactory.

In consideration of the great variation in parent material and age of the soils of the two regions on the one hand, and the uniformity of the soil organic matter conditions on the other, one is led to believe that the nitrogen equilibrium of cultivated soil responds sensitively to the operating climatic factors. Within large regions, having a wide range in climatic conditions, temperature, humidity, and vegetation apparently exert a greater influence on soil organic matter constituents of loam soils than does either parent material or age.

TABLE 3

Comparison of the organic matter temperature curve of the eastern United States with the nitrogen temperature curve of the Middle West

TEMPERATURE		CALCULATED NITROGEN CONTENT OF MID-WESTERN SOILS	CALCULATED ORGANIC MATTER CONTENT OF EASTERN SOILS	CALCULATED CARBON CONTENT OF EASTERN SOILS. *O.M.* ÷ 1.742	CARBON NITROGEN RATIO (C/N)
°*C.*	°*F.*	*per cent N*	*per cent O.M.*		
8	46.4	0.148	2.83	1.623	11.0
10	50.0	0.121	2.30	1.321	10.9
12	53.6	0.098	1.87	1.073	10.9
14	57.2	0.080	1.52	0.872	10.9
16	60.8	0.066	1.23	0.706	10.7
18	64.4	0.054	1.00	0.574	10.6
20	68.0	0.044	0.81	0.465	10.6
Average					10.8

SUMMARY[2]

The organic matter content of surface soils of 18 counties in the eastern United States was correlated with annual temperature. It was found that with *increasing* temperature, the organic matter content of the soil *decreases* exponentially.

[2] *Note during print:* In a recent paper on "Some carbon-nitrogen relations in soils" (*Soil Sci.* 30: 257–266, 1930) W. R. Leighty and E. C. Shorey published a series of nitrogen and carbon analyses of cultivated soils from the eastern United States. The data for timbered upland soils of loam and sandy loam texture show a pronounced decrease of nitrogen and organic matter from north to south as seen from the following table:

REFERENCES

(1) JENNY, H. 1929 Relation of temperature to the amount of nitrogen in soils. *Soil Sci.* 27: 169–188.

(2) JENNY, H. 1930 The nitrogen content of the soil as related to the precipitation-evaporation ratio. *Soil Sci.* 29: 193–206.

(3) JENNY, H. 1930 An equation of state for soil nitrogen. *Jour. Phys. Chem.* 34: 1053–1057.

(4) U. S. DEPARTMENT OF AGRICULTURE. Field operations of the Bureau of Soils 1902 and 1903.

(5) WAKSMAN, S. A. 1929 Chemical nature of soil organic matter, methods of analyses, and the rôle of micro-organisms in its formation and decomposition. *Trans. Second Comn. Internatl. Soc. Soil Sci.* (Budapest). A: 172–197.

FOOTNOTE 2—*continued*

Nitrogen-organic matter-temperature relationship in Eastern United States, after data from Leighty and Shorey

STATE	NUMBER OF ANALYSES	APPROXIMATE ANNUAL TEMPERATURE	N	ORGANIC MATTER (C × 1.724)
		°C.	*per cent*	*per cent*
Maine	1	4.0	0.227	5.17
Pennsylvania	4	11.0	0.176	3.12
New York	4	11.2	0.116	2.78
New Jersey	4	11.5	0.125	2.31
Virginia	7	15.0	0.074	1.36
North Carolina	6	16.0	0.047	1.42
South Carolina	4	17.0	0.028	0.92
Georgia	4	18.5	0.044	1.15
Florida	1	20.0	0.041	1.74

SOIL FORMING PROCESSES IN THE HAWAIIAN ISLANDS FROM THE CHEMICAL AND MINERALOGICAL POINTS OF VIEW

HAROLD S. PALMER
University of Hawaii

Received for publication June 1, 1930

The chemical composition of a soil depends on the nature of the original rock from which the soil is derived, on the nature of the weathering processes, and on the length of the weathering. Some idea of the nature of the processes may be had by comparing the chemical analyses of the original fresh rock and the derived weathered soil.

It is known, in a general way, that the chief effects of weathering in Hawaii are the conversion of aluminum and iron to hydrous oxides and the removal in solution of much of the other constituents.

It is also possible to compare the minerals of the original fresh rock with the minerals of the derived weathered soil. Both sorts of comparisons are undertaken in the present paper.

BASIC DATA

In order to study such processes one would wish for pairs of analyses of the fresh rocks and of the soils derived from them. Such data are lacking. Over a hundred superior analyses of Hawaiian basalts have been made, but the corresponding soil analyses are lacking. Although several hundred soil analyses are available, they were unfortunately made by the inferior process of digestion with hydrochloric acid, which leaves much undetermined matter in the "insoluble residue." One might compare the average of a large number of soil analyses, if superior ones were available, with the average of the rock analyses, but this would be open to the objection that one could not be sure that they represented the same material in the rock and in the soil condition. Moreover, such averages would integrate the effects of a variety of climatic types.

The best data available for such a study are the four pairs of analyses made by Kelley[1] on the fresh cores and on the weathered shells of four spheroidal boulders from gulches in the Wahiawa region of Oahu. Since all refer to one rather small locality they also show the effects of a single type of climate.

[1] KELLEY, W. P. 1912. The function and distribution of manganese in soils and plants, Hawaii Agr. Exp. Sta., Bul. 26. A typographical error in one of the analyses is corrected in bulletin 42 of the same series, Composition of Hawaiian Soil Particles, by W. T. McGeorge, 1917.

These analyses report the amounts of 13 radicals, and appear to have been made by the superior method of complete fusion with sodium carbonate. These pairs of analyses have the advantage that in each pair there has been no admixture of mineral or organic matter from foreign sources, consequently the weathered shells show precisely what happens to materials having the composition of the core. They have the disadavantage that the soil-forming process has not been carried to completion, but they do show the direction in which weathering is working. Another disadvantage, namely, that they represent only one locality and only one type of Hawaiian climate, is counterbalanced by the definiteness for that one locality and climatic type.

TABLE 1

Analyses of rock cores and weathered shells

RADICAL	A	B	C	D	E	F	G	H	I	J
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
SiO_2	52.45	20.29	52.15	24.01	51.98	26.82	52.24	32.00	52.21	25.78
Al_2O_3	11.49	37.97	12.57	36.27	15.85	30.13	16.00	35.28	13.98	34.91
Fe_2O_3	3.66	15.01	3.36	14.29	2.90	16.86	3.73	11.80	3.41	14.49
FeO	6.90	3.22	7.07	3.31	6.84	3.03	5.89	1.53	6.63	2.77
Mn_3O_4	0.36	0.19	0.50	0.43	0.92	0.06	0.68	0.08	0.62	0.19
MgO	5.81	0.20	6.51	0.09	5.61	0.11	5.90	0.14	5.96	0.14
CaO	10.32	0.33	8.54	0.17	9.57	0.22	9.54	0.22	9.49	0.24
K_2O	0.89	0.25	0.84	0.24	0.97	0.46	0.86	0.30	0.89	0.31
Na_2O	2.44	0.27	2.64	0.31	2.70	0.57	2.65	0.61	2.61	0.44
SO_3	0.20	0.78	0.61	0.49	0.51	0.74	0.53	0.70	0.46	0.63
P_2O_5	0.38	0.23	0.28	0.34	0.22	0.19	0.11	0.04	0.25	0.20
TiO_2	4.07	4.69	4.07	4.84	1.50	2.21	1.50	2.13	2.79	3.47
H_2O	1.02	16.84	0.94	15.61	1.04	18.34	0.54	15.06	0.89	16.46
Sum	99.99	100.27	100.08	100.40	100.61	99.74	100.17	99.89	100.19	100.03

A, C, E, and G—Analyses of the four fresh cores of spheroidal boulders.
B, D, F, and H—Analyses of the four corresponding weathered shells.
I—Average of A, C, E, and G.
J—Average of B, D, F, and H.

The nearest station for which climatological data are available is Upper Hoaeae, which has a mean annual rainfall of 34.50 inches. The mean annual temperature is 71.8°F. The warmest month (August) averages 75.6° and the coldest month (January) averages 67.7°F. A standard U. S. Weather Bureau evaporation pan shows an evaporation of about 61 inches a year.

The four pairs of analyses, as reported by McGeorge, are given in table 1, together with averages of the four pairs.

CHEMICAL INTERPRETATION

The fresh, original rock and the weathered derived material can be compared in two ways. Since rocks consist of aggregations of grains of one or more

minerals, the minerals of the rock can be compared with the minerals of the derived material, as one of the methods. The minerals, however, are chemical compounds, and a given element is likely to exist in more than one of the minerals of a rock. Chemical analyses show the amounts of the various radicals in the rock. The second method, then, is to compare the chemical analyses. This will be undertaken first.

In table 2 the averages of table 1 (I and J) are recast so as to total 100 per cent for use in subsequent computations, and also so as to convert Mn_3O_4 to

TABLE 2

Chemical changes during weathering

RADICAL	K	L	M	N	O	P	Q	R
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
SiO_2	52.13	25.77	10.31	−41.82	......	−80.2	....	19.8
Al_2O_3	13.96	34.90	13.96	0.00	0.00	......	....	100.0
Fe_2O_3	3.40	14.49	5.80	....	+2.40	......	+70.3	170.3
FeO	6.62	2.77	1.11	−5.51	......	−83.2	....	16.8
MnO	0.58	0.18	0.07	−0.51	......	−87.9	....	12.1
MgO	5.95	0.14	0.06	−5.89	......	−99.0	....	1.0
CaO	9.47	0.24	0.10	−9.37	......	−98.9	....	1.1
K_2O	0.89	0.31	0.12	−0.77	......	−86.5	....	13.5
Na_2O	2.61	0.44	0.18	−2.43	......	−93.1	....	6.9
SO_3	0.46	0.63	0.25	−0.21	......	−45.7	....	54.3
P_2O_5	0.25	0.20	0.08	−0.17	......	−68.0	....	32.0
TiO_2	2.79	3.47	1.39	−1.40	......	−50.2	....	49.8
H_2O	0.89	16.46	6.58	....	+5.69	......	+640.0	728.1
Sum	100.00	100.00	40.01	−68.08	+8.09	......	....	.....

K—Recast average of four fresh cores.

L—Recast average of four weathered shells.

M—Preceding recast on the assumption of no change of Al_2O_3. Equals $L \times 0.4000 = L \times \frac{13.96}{34.90}$.

N and O—Losses and gains by weathering in terms of percentage of total fresh cores. Equals M minus K.

P and Q—Losses and gains in terms of percentage of the amount of each radicle originally present. Equals N (or O)/K $\times$ 100.

R—Amount of each radicle remaining in terms of percentage of amount of each radicle originally present. Equals M/K $\times$ 100.

the equivalent amount of MnO, the more probable form in which manganese enters silicate minerals.

For the present study the averages of the four pairs of analyses are used, since a preliminary study showed that the behavior of each pair was much like the behavior of the other pairs and of the averages.

The arbitrary assumption, in computing column M from columns K and L of table 2, that there has been neither gain nor loss of alumina (Al_2O_3) in weathering, deserves a little discussion. Of the various radicals present, water is

the only one for which we can conceive a source that might supply additions. Atmospheric oxygen also is undoubtedly added and accounts for the apparent increase of ferric oxide (Fe_2O_3)—much of the ferrous oxide (FeO) is oxidized to ferric oxide. There are no conceivable sources for additions the other radicals. Columns K and L show that, excepting water and ferric oxide, alumina has the highest ratio between its amount in the weathered shells and its amount in the fresh cores. This indicates that alumina has suffered the least loss. It is very probable that natural Hawaiian waters contain very little alumina, but the available water analyses give no evidence on this point. They report alumina, if they report it at all, along with ferric oxide, with which it is precipitated and weighed in the usual routine. Since some assumption must underlie the computations, and since the loss of alumina is the smallest, the selected assumption is that there has been no loss or gain of alumina. On this assumption, it appears that 100 parts by weight of the original fresh rock lose about 67 parts by solution (column N) and gain about 6 parts of water by hydration and 1 parts of oxygen by oxidation of ferrous to ferric oxide. Thus, 100 parts of fresh rock give rise to 40 parts of the weathered material. (The total for column N is about 68 whereas the loss by solution just given is 67. The total for column O is about 8, whereas the gains of water and oxygen just given total 7. These discrepancies are due to the shifting of about 1 part of iron from the ferrous to the ferric state.)

Similar studies of other soils have indicated that titania is the most stable constituent. Such is not the case with the analyses here studied. Computation on the assumption of no change of titania would demand greater increases of water and of ferric oxide, and would also demand that the amount of alumina be doubled during the weathering and the amount of sulfur trioxide be increased nearly 11 per cent. It is conceivable that sulfur trioxide might be derived from the gypsum in wind-blown spray, but there is no conceivable source for even a small part of the large increase of alumina demanded. Titania therefore, is less stable than alumina.

It is probable that if oxidizing conditions prevailed throughout the weathering process, iron would be as stable as alumina. Under reducing conditions, however, iron is less stable than alumina. Leith and Mead[2] state on the basis of their studies of the lateritic iron ores of Cuba and the bauxitic aluminum ores of the southern states, that basic rocks under reducing conditions lose iron and give rise to bauxite and gibbsite, hydrous oxides of aluminum. Under moist conditions, with much vegetation to produce reducing conditions, this process removes color producing iron oxides and so gives rather bleached soils in places in Hawaii. Under oxidizing conditions the end product of the weathering of basic rocks is laterite. Leith and Mead ascribe the proverbially rapid weathering of basic rocks to the chemical instability of the lime feldspars, of the pyroxenes, and of the olivines which weather and are dissolved out, causing

[2] Leith, C. K., and Mead, W. J. 1915. Metamorphic Geology, p. 37, Henry Holt & Co.

a porous texture which allows ground water to enter readily and attack other mineral grains.

If the various radicals are considered in the order of decreasing gains, or increasing losses, as compared to the amount of the various radicals in the fresh, original rock, the relative susceptibility of the various radicals is shown.

The following may be said in summarizing the chemical changes:

Water is added in large amounts and is an actual addition.

Oxygen is actually added, and explains the apparent addition of ferric oxide.

The lack of any change in alumina, though due to an arbitrary assumption, indicates that alumina is the most stable radical in the list.

Sulfur trioxide and titania decrease to amounts between three-eights and five-eights of the original amounts.

Phosphorus pentoxide, silica, ferrous oxide, and potash decrease to amounts between one-eighth and three-eighths of the original amounts. Manganous oxide falls just a little outside this group.

Manganous oxide and soda decrease to amounts between one-sixteenth and one-eighth of the original amounts.

Lime and magnesia decrease to about one-hundredth of the amount originally present.

A check might be had on the foregoing conclusions as to the relative amounts of the various radicals removed during the weathering if we could analyze the waters that did the removing. This is obviously impossible, but we can examine the character of other Hawaiian natural waters. The best data available to me for such a check are given by a single analysis of water collected from Kalihi Stream, at an elevation of about 470 feet, on August 16, 1928.

Though these data are the best available at the present moment, it must be remembered that they represent a water from a point removed from the water that weathered the rock by a distance of some 15 miles and by a time that is to be measured in hundreds of years. Moreover the two regions have somewhat different climates and decidedly different topographic characters. As a consequence too close correspondence between them is not to be expected.

The analysis of the Kalihi water is given in part in column Y of table 3. The bicarbonate and nitrate radicals are omitted since they are not included in the rock analyses. The ingredients of this water are in part derived by solution from the rocks and in part from wind-blown sea salt. It is necessary, therefore, to subtract from the total the part derived from the sea, in order to learn what has been derived from the rocks. Column W of table 3 gives the composition of the dissolved matter in sea water. These are adjusted by multiplying by 16/56.292, which brings the value for chlorine to the amount of chlorine in the Kalihi Stream water. Chlorine is used as a basis for this conversion since it is reasonable to assume that none of the chlorine in the Kalihi water is derived from the rocks. By a further adjustment the elemental ingredients reported in the sea water are converted to the equivalent oxides for comparison with the oxides removed from the rock. After this double adjustment the amounts of the salts supplied from sea water to the Kalihi water are those given in column X, table 3.

Column Y of this table gives the analysis of the Kalihi water as reported, and column Z gives this analysis with the elemental ingredients converted to the equivalent oxides. Subtracting the values in column X from those in

TABLE 3

Comparison of materials dissolved in Kalihi stream water with materials lost in weathering as previously calculated

RADICAL	W	X	Y	Z	AA	BB	CC	DD
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
SiO_2	0.000	0.000	26.00	26.00	26.000	59.81	41.82	65.13
Al_2O_3	0.000	0.000	.....	.....	0.000	0.00	0.00	0:00
Fe	0.000	0.000	0.43	......	......	......	......	......
Fe_2O_3	......	......	.....	0.61	0.61	1.40	3.72	5.79
Mg	3.725	......	3.8	.....	......	......	.....	......
MgO	......	1.787	.....	6.30	4.513	10.38	5.89	9.17
Ca	1.197	......	4.2	.....	......	......	.....	......
CaO	......	0.865	.....	5.88	5.015	11.54	9.37	14.59
Na	30.593	......	12.0	.....	......	......	.....	......
Na_2O	......	11.933	.....	16.17	4.241	9.76	2.43	3.78
K	1.106	......	0.9	.....	......	......	.....	......
K_2O	......	0.386	.....	1.08	0.694	1.60	0.77	1.20
SO_4	7.692	......	5.1	.....	......	......	.....	......
SO_3	......	1.855	.....	4.25	2.395	5.51	0.21	0.33
Cl	55.222	16.000	16.0	16.00	0.000	0.00	.....	......
				76.29	43.468	100.00	64.21	100.00

W—Average composition of salts in 77 sea water samples. F. W. Clarke, The Data of Geochemistry, Ed. 4, p. 123, U. S. Geol. Survey, Bull. 695.

X—Same adjusted to oxides instead of elements, and to make chlorine equal to the chlorine in the Kalihi water (column Y).

Y—Composition of Kalihi Stream water (Personal Communication).

Z—Same, adjusted to oxides instead of elements.

AA—Composition of salts in Kalihi water after deducting those derived from salt spray (equals Z-X).

BB—Same as AA, adjusted to total 100.

CC—Material removed in weathering rocks.

DD—Same as CC, adjusted to total 100 for comparison with column BB.

column Z, we get column AA. This is equivalent to subtracting from the Kalihi water the ingredients supplied from sea water so that the remainder, column AA, is the material dissolved from the rocks and soil by the Kalihi water since it fell as rain.

Column CC, repeats columns N and O of table 2, except that the gain of Fe_2O_3 and the loss of FeO are balanced off in terms of like Fe, the net change being expressed as the equivalent Fe_2O_3. Column CC represents the losses as obtained by comparing the fresh and weathered materials.

Column AA totals 43.468 and column CC totals 64.21, and are therefore a little difficult to compare. Comparison would be facilitated if both were adjusted to the same total. In columns BB and DD the data of columns AA and CC, respectively, have been multiplied by suitable factors so that both total 100.

Columns BB and DD, show that there is rather close agreement as to silica, magnesia, lime, and potash; but that there is less good correspondence for ferric oxide, soda, and sulfate. It would appear that the Kalihi water has picked up sodium sulfate in some unexplained way, and has lost some ferric oxide.

MINERALOGICAL INTERPRETATION

The calculation of the mineral composition of a rock from its chemical analysis is often performed by petrologists. The percentages by weight of each radical are divided by the corresponding radical weights. The quotients thus obtained give the relative numbers of the radicals present, which are called the "radical ratios." These are then distributed to form mineral molecules in accordance with certain established standard procedures. The numbers of molecules of each mineral are then multiplied by the molecular weights of the minerals, and the products are the percentages of the minerals in the rock. The standard procedure among petrologists gives comparable results, and the mineral composition thus arbitrarily computed is known as the "norm" of the rock. The norm usually differs from the "mode" or actual mineral composition of the rock.

The averages of the four fresh cores and of the four weathered shells (columns I and J, table 1) are thus treated in tables 4 and 6, with the exception that the standard procedure has been modified in order to make the computed composition more like the known mode of the Hawaiian basalts.

In explanation of the computations, we might consider the treatment of the ferrous oxide and the titania in table 4. The adjusted percentages of these radicals are 6.62 and 2.79, and their radical weights are 71.84 and 80.1, respectively. Dividing the percentages by the radical weights we get $3.40/71.84 = 0.0921$ and $2.79/80.1 = 0.0348$. The quotients are multiplied by 10,000 in order to give integers, which are more convenient to handle. We may say, then, that for every 921 radicals of ferrous oxide there are 348 radicals of titania. There is a mineral in Hawaiian basalts called "ilmenite" whose molecule ($FeO \cdot TiO_2$ or $FeTiO_3$) consists of one radical of ferrous oxide and one radical of titania. So the 348 radicals of titania and 348 of the 921 radicals of ferrous oxide are combined to make 348 molecules of ilmenite. In the lower part of table 4, the 348 molecules of ilmenite again appear, where they are multiplied

by 151.94, the molecular weight of ilmenite, giving a product of 528.7, which by a rounding off and shifting of the decimal point shows that the rock contains 5.29 per cent of the mineral ilmenite.

After 348 radicals of ferrous oxide have been set aside for ilmenite 573

TABLE 4

Computation of norm of average of four fresh cores

RADICAL	PER CENT	RADICAL RATIO	Il.	Ap.	No.	Or.	Ab.	An.	Ma.	METASILICATES				Qz.
										Fe	Ca	Mg	Mn	
SiO_2	52.13	8,680	...	...	228	564	1,500	1,822	...	360	723	1,476	82	1,925
Al_2O_3	13.96	1,369	...	...	114	94	250	911	...	...	...	...	...	...
Fe_2O_3	3.40	213	...	...	...	...	...	...	213	...	...	...	...	...
FeO	6.62	921	348	...	...	...	...	...	213	360	...	...	...	...
MnO	0.58	82	...	...	...	...	...	...	...	...	...	...	82	...
MgO	5.95	1,476	...	...	...	...	...	...	...	...	...	1,476	...	...
CaO	9.47	1,688	...	54	...	...	...	911	...	...	723	...	...	...
K_2O	0.89	94	...	...	...	94	...	...	...	...	...	...	...	...
Na_2O	2.61	421	...	...	171	...	250	...	...	...	...	...	...	...
SO_3	0.46	57	...	...	57	...	...	...	...	...	...	...	...	...
P_2O_5	0.25	18	...	18	...	...	...	...	...	...	...	...	...	...
TiO_2	2.79	348	348	...	...	...	...	...	...	...	...	...	...	...
H_2O	0.89													
	100.00													

MINERAL	COMPOSITION	NUMBER OF MOLECULES	MOLECULAR WEIGHT	PER CENT
Ilmenite	$FeOTiO_3$	348	151.94	5.29
Apatite	$Ca_3P_2O_8 + 1/3CaF_2$	18	336.34	0.61
Noselite	$Na_8Al_6Si_6SO_{16}$	57	710.12	4.05
Orthoclase	$KAlSi_3O_8$*	94	556.49	5.23
Albite	$NaAlSi_3O_8$*	250	524.28	13.11
Anorthite	$CaAl_2Si_2O_8$	911	278.15	25.34
Magnetite	Fe_3O_4	213	231.52	4.93
Iron metasilicate	$FeSiO_3$	360	131.90	4.75
Wollastonite	$CaSiO_3$	723	116.15	8.40
Clinoenstatite	$MgSiO_3$	1,476	100.38	14.82
Rhodonite	$MnSiO_3$	82	130.99	1.07
Quartz	SiO_2	1,925	60.06	11.56
Water	H_2O	.....	......	0.89
				100.05

* This is half the formula used in the upper part of the table.

remain. Of these, 213 are next set aside to match the 213 radicals of ferric oxide to make magnetite ($FeO \cdot Fe_2O_3$ or Fe_3O_4), leaving 360 to be combined with part of the silica for iron metasilicate ($FeO \cdot SiO_2$ or $FeSiO_3$).

The order in which the radicals are set aside to form various minerals is essentially that which the study of countless rocks has shown to be the order

of crystallization in nature. The relative affinities of the various kinds of radicals for one another are well known for the igneous rocks, but not for the weathered products.

In the fresh cores, all the titania and a proper amount of ferrous oxide are first assigned to ilmenite, which is one of the first minerals to crystallize. Next, enough lime is assigned to the available phosphorus pentoxide to form apatite, also an early forming mineral. The sulphur trioxide surely exists as noselite, or some related mineral. Therefore, suitable amounts of soda, alumina, and silica are assigned to the sulfur trioxide for noselite. At this stage all of the acids, except silica, have been used up. Silica is first assigned, along with proper amounts of alumina, to the strong bases to form the potash and soda feldspars (orthoclase and albite). So far as alumina is still available, the lime feldspar (anorthite) is made, which takes part of the lime and silica. With alumina all gone, the remaining silica is used as far as necessary to make metasilicates of ferrous oxide, lime, magnesia, and manganous oxide. In the preceding discussion one step has been omitted, namely, the assigning of part of the ferrous oxide to the ferric oxide for magnetite. Finally, after all the bases have been assigned, there still remains some silica, which is taken for quartz. In the calculations, the metasilicates are considered independently, but they actually do combine as dual or even trial metasilicates, thus forming a complex group of minerals known as the pyroxenes. Were there not enough silica to go around among the bases, as metasilicates, we would first have satisfied the lime and manganous oxide. Then in accordance with suitable formulas, we would have made partly metasilicates (pyroxenes) and partly orthosilicates (olivines) with the magnesia and ferrous oxide.

The pyroxenes are a series of related metasilicates which usually have lime, magnesia, and ferrous oxide as their basic radicals. The oxides of manganese, the alkalies, zinc, aluminum, and ferric iron form the bases in some rarer varieties. Some are characterized by having a single base: for example, enstatite, which is $MgO \cdot SiO_2$. (This differs from the corresponding orthosilicate, forsterite, $2MgO \cdot SiO_2$, in having twice as much silica in proportion to the magnesia.) Most pyroxenes are either compounds or mixtures of silicates of two or more bases, for example, diopside, which is $MgCaSi_2O_6$. Many pyroxenes are far more complicated. Washington and Merwin[3] give an analysis (table 5) of an augite, a variety of pyroxene, from near the summit of Haleakala.

According to Washington and Merwin the "augite is thus essentially a hedenbergite-diopside with small amounts of acmite, clinoenstatite, and alumina in solid solution."

Undoubtedly all the augites of Hawaii are of much the same complex character as this one.

[3] WASHINGTON, H. S., AND MERWIN, H. E. 1922. Augite of Haleakala, Maui, Hawaiian Islands. *Amer. Jour. Sci.* 3: 117–122.

A rather striking thing in the norm of the fresh cores, given in table 4, is the occurrence of quartz. Quartz does not occur as a primary mineral in the basalts of Hawaii, and yet it is shown in the norm. The probable explanation is that the quartz is present in solid solution in the glass that fills the interstices between the crystalline grains of the other minerals. In the normal sequence of crystallization of various minerals out of a magma, quartz is among the very last to form. It is therefore likely to remain non-crystalline or glassy when cooling, finally brings about the rigid state of glass in the residual melt. Minerals richer in silica than those used in the preceding calculation of the norm are not to be found by microscopic examination of thin sections of Hawaiian rocks.

The steps in calculating the mineral composition of the weathered shells are rather similar, and are set forth in table 6, but the choice of an association

TABLE 5

Chemical analysis and calculated mineral composition of the augite of Haleakala

RADICAL	PER CENT	MINERAL	FORMULA	PER CENT
SiO_2	47.70			
Al_2O_3	6.82			
Fe_2O_3	3.36			
FeO	4.43	Diopside	$CaMgSi_2O_6$	69.12
MnO	0.16	Hedenbergite	$CaFeSi_2O_6$	15.13
MgO	13.34	Acmite	$NaFe^{III}Si_2O_6$	5.08
CaO	21.35	Clinoenstatite	$MgSiO_3$	1.90
K_2O	0.03	Iron metasilicate	$FeSiO_3$	0.40
Na_2O	0.65	Corundum and Hematite	$(Al, Fe)_2O_3$	8.65
TiO_2	1.89			
Cr_2O_3	0.23			100.28
H_2O	0.15			
	100.11			

of minerals is not controlled by anything corresponding to the known order of crystallization used for the fresh cores. Perhaps it would be impossible to establish a proper choice of minerals, as the grains are so small that they might not be identifiable under the microscope.

In the absence of guiding experience, the process used as is follows:

The phosphorus pentoxide is first taken to make apatite, which incidentally leaves very little lime for future assignment to gypsum, a known constituent of some Hawaiian soils. Next, titania is taken to satisfy the available ferrous oxide for ilmenite, as before, the residue of titania being calculated as rutile. The small amount of manganous oxide is given enough of the large amount of water to form psilomelane. At this stage of the computation the only acids remaining are silica and sulfur trioxide. The sulfur trioxide is then used to make the hydrous sulfates of calcium and magnesium, gypsum, and epsomite. To the small amount of sulfur trioxide remaining is assigned part of the soda for mirabilite, the hydrous sulfate of sodium. Now only silica remains of the acids. To the potash and the remaining soda are assigned proper amounts of the ample silica and alumina to make the feldspars, orthoclase,

and plagioclase. (No lime is available for making more stable minerals.) There now remain only water, ferric oxide, alumina, and silica. To the ferric oxide is given enough water to make limonite. The remaining water is used to make gibbsite, the more hydrous, and bauxite, the less hydrous, both of which are hydrous oxides of aluminum. Finally, the remaining silica is computed as quartz.

TABLE 6

Calculation of the mineral composition of the weathered shells

RADICAL	PER CENT	RADICAL RATIO	Ap	Il.	Ru.	Ps.	Gy.	Ep.	Mi.	Or.	Ab.	Li.	Ba.	Gi.	Qz.
SiO_2	25.77	4,292	..	...	..	..	.	...	...	198	168	.....	......	...	3,926
Al_2O_3	34.90	3,423	..	...	..	..	.	...	...	33	28	.....	3,039	323	.....
Fe_2O_3	14.49	908	..	...	..	..	.	...	...	...	...	908	......	...	.....
FeO	2.77	386	..	386	...	..	.	...	...	...	...	.....	......	...	.....
MnO	0.18	25	..	...	..	25	.	...	...	...	...	.....	......	...	.....
MgO	0.14	35	..	...	..	..	.	35	...	...	...	.....	......	...	.....
CaO	0.24	43	42	...	..	..	1	...	...	...	...	.....	......	...	.....
K_2O	0.31	33	..	...	..	..	.	...	...	33	...	.....	......	...	.....
Na_2O	0.44	71	..	...	..	..	.	...	43	...	28	.....	......	...	.....
SO_3	0.63	78	..	...	..	..	.	35	43	...	...	.....	......	...	.....
P_2O_5	0.20	14	14	...	..	..	.	...	...	...	...	.....	......	...	.....
TiO_2	3.47	433	..	386	47	..	.	...	...	...	...	.....	......	...	.....
H_2O	16.46	9,136	..	...	..	50	2	245	430	...	...	1,362	6,078	969	.....

MINERAL	COMPOSITION	NUMBER OF MOLECULES	MOLECULAR WEIGHT	PER CENT
Apatite	$Ca_3P_2O_8 + 1/3CaF_2$	14	336.34	0.47
Ilmenite	$FeTiO_3$	386	151.94	5.86
Rutile	TiO_2	47	80.1	0.38
Psilomelane	H_4MnO_5	25	138.962	0.35
Gypsum	$CaSO_4 \cdot 2H_2O$	1	172.86	0.02
Epsomite	$MgSO_4 \cdot 7H_2O$	35	246.496	0.86
Mirabilite	$Na_2SO_4 \cdot 10H_2O$	43	322.204	1.39
Orthoclase	$KAlSi_3O_8$*	33	556.49	1.84
Albite	$NaAlSi_3O_8$*	28	524.28	1.47
Limonite	$2Fe_2O_3 \cdot 3H_2O$†	908	186.704	16.95
Bauxite	$Al_2O_3 \cdot 2H_2O$	3,039	137.972	41.93
Gibbsite	$Al_2O_3 \cdot 3H_2O$	323	155.988	5.04
Quartz	SiO_2	3,926	60.06	23.58
				100.14

* These formulas give half the molecular weight used in the upper part of the table.

† This formula gives double the molecular weight used in the upper part of the table.

The abundance of the various minerals in the fresh rock and in the weathered shells is given in table 7.

Comparison of the last column (GG) with the first column (EE) shows that the removal of material by solution has been the chief process in action. This

TABLE 7

Comparison of the mineral compositions of the fresh cores and of the weathered shells

GROUP	MINERAL*	EE	FF	GG
		per cent	*per cent*	*per cent*
Baseless mineral	Quartz	11.56	23.58	9.43
Titania minerals	Ilmenite	5.29	5.86	2.34
	Rutile	......	0.38	0.15
Potash mineral	Orthoclase	5.23	1.84	0.74
Soda minerals	Noselite	4.05	......	.....
	Albite	13.11	1.47	0.59
	Mirabilite	......	1.39	0.56
Lime minerals	Apatite	0.61	0.47	0.19
	Anorthite	25.34	......	.....
	Wollastonite	8.40	......	.....
	Gypsum	......	0.02	0.01
Magnesia minerals	Clinoenstatite	14.82	......	.....
	Epsomite	......	0.86	0.34
Iron minerals	Iron metasilicate	4.75	......	.....
	Magnetite	4.93	......	.....
	Limonite	......	16.95	6.78
	Ilmenite†			
Aluminum minerals	Feldspars‡			
	Gibbsite	......	5.04	2.02
	Bauxite	......	41.93	16.77
Manganese minerals	Rhodonite	1.07	......	.....
	Psilomelane	......	0.35	0.14
	Water	0.89	......	.....
		100.05	100.14	40.06

* This column groups together minerals with the same bases.

† Ilmenite is also a titania mineral and is listed above.

‡ The feldspars (orthoclase, albite, and anorthite) are listed above with the potash, soda, and lime minerals.

EE—Mineral composition of the fresh rock. The data of the last column of table 4, but in different order.

FF—Mineral composition of the weathered shells. The data of the last column of table 5, but in different order.

GG—Mineral composition of the weathered shells reduced to show the amounts surviving or newly generated. Equals FF $\times \frac{40.06}{110.14}$. This computation assumes no change in alumina.

is shown by inspection for the minerals having titania, potash, soda, lime, magnesia, or manganous oxide as their bases, as well as by the total of all constituents. It is also shown immediately for apatite and quartz. At the first glance it might appear that alumina has been added, but it must be remembered that the alumina in the gibbsite and bauxite is not new alumina, but is derived from the destruction of the three feldspars, orthoclase, albite, and anorthite.

TABLE 8

Parental relationships of original minerals of fresh cores to derived and surviving minerals of weathered shells

PARENT MINERALS	DERIVED AND SURVIVING MINERALS												
	Apatite	Ilmenite	Rutile	Psilomelane	Gypsum	Epsomite	Mirabilite	Orthoclase	Albite	Limonite	Bauxite	Gibbsite	Quartz
Apatite	x	...	...	...	...	...	...	...	...	...	...	...	...
Ilmenite	...	x	x	...	...	...	...	...	...	x	...	...	...
Noselite	...	...	...	...	x	x	x	...	...	...	x	x	x
Orthoclase	...	...	...	...	...	...	...	x	...	...	x	x	x
Albite	...	...	...	...	...	...	x	...	x	...	x	x	x
Anorthite	...	...	...	...	x	...	...	...	...	...	x	x	x
Magnetite	...	...	...	...	...	...	...	...	...	x	...	...	...
Iron metasilicate	...	...	...	...	...	...	...	...	...	x	...	...	x
Wollastonite	...	...	...	...	x	...	...	...	...	...	...	...	x
Clinoenstatite	...	...	...	...	...	x	...	...	...	...	...	...	x
Rhodonite	...	...	...	x	...	...	...	...	...	...	...	...	x
Quartz	...	...	...	...	...	...	...	...	...	...	...	...	x

Table 8 attempts to set forth the genetic relationships of the derived minerals to the parent minerals. Since parenthood cannot be definitely ascribed, only probabilities are indicated, which is done by the use of *X* marks. No further discussion of table 8 seems necessary.

FURTHER EVIDENCE CONCERNING THE TOXIC ACTION OF ALUMINUM IN CONNECTION WITH PLANT GROWTH[1]

BASIL E. GILBERT AND FREDERICK R. PEMBER

Rhode Island Agricultural Experiment Station

Received for publication June 23, 1930

For many years the Rhode Island Agricultural Experiment Station has studied acid-soil conditions. From time to time various aspects of the problem have been explored and the publications of the station show the results of much experimentation. "Active" soil aluminum has been linked with acidity as measured by hydrogen-ion concentration and lime requirement. Much of the work has been based on solution cultures using various plants and amounts of aluminum at different acidities.

Other workers have added to the mass of information now available and two schools of thought have arisen. Line (6) in England has claimed that aluminum cannot exist in the soil solution in sufficient concentration to cause toxicity and that the toxic factor of acid soils is acidity as measured by hydrogen-ion concentration. McLean (8) has shown that aluminum may be toxic to plants in solution culture in amounts far smaller than those found by Line in the soil solutions.

This article describes attempts to clarify the problem by endeavoring to divorce the two factors, acidity and aluminum, and to show by solution culture methods accompanied by the study of certain acid soils from widely separated areas and having differing geological histories, which of the two factors is the more potent.

SOLUTION CULTURES

The indicator plant selected for this work was Cos lettuce. The seedlings were germinated in beach sand and when approximately 1 week old were sorted for uniformity, and placed in corks with their roots immersed. Six plants were placed in each culture and all cultures were in quadruplicate. After 7 days the solution was renewed, and the plants were harvested at the end of 14 days. Colorimetric determinations of pH values were made at the beginning and end of the experiment. The plants were carefully dried in a constant temperature oven, and after they had come to equilibrium with room conditions, air-dry weights were obtained.

[1] Contribution no. 395 of the R. I. Agricultural Experiment Station.

Table 1 gives the ingredients in 1,000 cc. of the culture solutions used in this experiment. Each culture bottle contained 250 cc. of solution.

In table 2 are reported the yields of seedlings grown in the six different culture solutions.

From table 2 it will be noted that although the reactions during the growth period varied from pH 3.2 to 7.5, no appreciable differences in yields are

TABLE 1

Ingredients in 1 liter of culture solutions used with lettuce seedlings

Solution Number		I	II	III	IV	V	VI
	MOLARITY	MODIFIED KNOPS	R. I. SOLUTION B	PFEFFERS' SOLUTION	R. I. SOLUTION A	R. I. SOLUTION A, DOUBLED	R. I. SOLUTION A, TREBLED
	M	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
$Ca(NO_3)_2 \cdot 4H_2O$	0.100	19	15	40	15	30	45
KH_2PO_4	0.030	8	....	10	..	..	..
$MgSO_4 \cdot 7H_2O$	0.100	8	8	5	8	16	24
$Fe_2(NO_3)_6 \cdot 18H_2O$	0.003	4	4	4	4	8	12
KCl	0.100	..	8	5	8	16	24
$CaH_4(PO_4)_2 \cdot H_2O$	0.010	..	13.6	..	6	12	18
KNO_3	0.100	..	....	10	..	..	..
NH_4NO_3	0.100	..	....	..	10	20	30

TABLE 2

Dry-weight yields of Cos lettuce seedlings grown in nutrient solutions having reaction ranges from pH 3.2 to 7.5

NUTRIENT SOLUTION NUMBER	REACTION RANGE DURING GROWTH PERIOD	DRY-WEIGHT YIELD
	pH	*gm.*
I	5.4–7.5	0.413
II	5.4–7.4	0.447
III	5.2–7.3	0.406
IV	5.3–3.4	0.488
V	5.4–3.4	0.428
VI	5.3–3.2	0.506

noticeable. It would thus appear that in solution Cos lettuce seedlings are not very susceptible to injury from acidity as measured by reaction *per se*.

In order to link the preceding experiment with the effect of aluminum when added to the culture solution Cos lettuce seedlings were grown in solution. The check solution was the same as no. IV (table 1) except that no phosphoric acid was added. Sulfuric acid was added to this culture solution to cause a pH of 4.6 and this constituted the second solution. The third solution con-

sisted of the check solution with the addition of 28 p.p.m. of aluminum as aluminum sulfate.

The alternating solution method adopted by McLean and Gilbert (9) was used. The following alternations were made.

DATE	CULTURE
May 11	I R. I. Solution A but no P_2O_5
	II R. I. Solution A and H_2SO_4 but no P_2O_5
	III R. I. Solution A and $Al_2(SO_4)_3$ but no P_2O_5
May 14	R. I. Solution A with all cultures
May 18	Similar to May 11
May 22	Similar to May 14

Table 3, in which the average yields of lettuce resulting from the aforementioned three treatments are given, shows that with a reaction range of pH 5.3 to 3.6, induced either naturally by selective withdrawal of ions from solution by the plants or artifically by sulfuric acid, no material difference in yield is noted. The addition of 28 p.p.m. of aluminum, however, produced a distinct depression in growth.

TABLE 3

Dry-weight yields of Cos lettuce seedlings as affected by acidity and aluminum

NUTRIENT SOLUTIONS	REACTION RANGE	DRY-WEIGHT YIELD
	pH	*gm.*
R. I. Solution A*..	5.3–3.6	0.940
R. I. Solution + H_2SO_4..................................	4.6–3.7	0.901
R. I. Solution + $Al_2(SO_4)_3$................................	4.6–4.6	0 602

* No phosphoric acid was added to this solution during one-half of the alternations.

ACID SOILS

This portion of the work may well be considered an extension of work begun by Burgess (3) in 1923 to compare "active" aluminum and hydrogen-ion concentration of widely separated acid soils. Samples of acid soils were obtained from widely separated locations. In many cases these were duplicates of the soils used by Burgess. Reference is, therefore, made to the aforementioned publication for their description. In some cases it would seem that during the interval between Burgess' work and the obtaining of the 1928 samples some of the fields from which these samples were taken had received lime.

Certain of the soils used in this work and which were not described in the 1923 publication are listed in the following:

RHODE ISLAND.—*Merrimac Silt loam.* This soil was taken from plat 82 of the area devoted to field experiments at the Rhode Island Agricultural Experiment Station. This plat has been well fertilized for many years but has received no lime.

CONNECTICUT.—*Taugwank loam.* This sample was taken from an old mowing lot near Stonington. It has not been fertilized or manured for many years.

Hollis Silt loam. This soil was obtained from an old mowing lot near Woodbridge. No fertilizer or manure had been applied for 18 years.

ILLINOIS.—*Muscatine Silt loam.* This sample was taken in Champaign County. It is a friable medium-brown silt loam.

NEW JERSEY.—*Sassafras Silt loam.* This sample was taken from the college farm at New Brunswick. It is a sedentary soil and is derived from unconsolidated deposits of sand, gravel, and clay.

The chemical determinations,[2] the results of which are reported in table 4, were made according to proved and common methods. Soil reactions were determined by the quinhydrone electrode. The lime requirements were determined by the Jones calcium acetate method as described by Burgess (1), except that the results were calculated to the dry soil basis and that soil particles that failed to pass a 2-mm. sieve were discarded. "Active" aluminum as defined by Burgess (2) is that dissolved from 1 part of soil shaken for 1 hour with 5 parts of 0.5 *N* acetic acid. The samples used for this extraction were passed through a 5-mesh sieve. Aluminum in the extract was determined by a procedure based on the method recommended by Lundell and Hoffman (7). The results were calculated to the dry soil basis.

Manganese soluble in 0.5 *N* acetic acid was determined for all soils as recommended by Jacobson,[3] but since the greatest concentration found, 70 p.p.m. of dry soil, is considered too low to indicate manganese toxicity, the results were not included in the tabulation.

At the beginning of the physiological work, lettuce seedlings were chosen as being sensitive to soil acidity and "active" aluminum. Burgess and Pember (4) have shown lettuce to be very sensitive when grown in soil, and McLean and Gilbert (8) have placed the limits at which aluminum in solution was toxic to lettuce as 2 p.p.m. of added aluminum. Lettuce was grown in these soils, but was found too sensitive for the purpose. The dry weights were so small that they were not considered worthy of inclusion in this report. In fact with several of the soils no growth took place after the lettuce plants were set in the pots.

Thus it became necessary to choose a crop less sensitive, and, following the classification given by Burgess and Pember (4) of "comparative resistances of certain plants to soil acidity and active aluminum," barley was chosen. This crop proved to be more suitable.

For this study Wagner pots were used and approximately 6,000 gm. of dry soil was weighed into each pot. Each soil was studied in duplicate. The water-holding capacity of the soils was determined and the moisture made up to 60 per cent of this figure. After the soils had stood for 14 days in the moist state, a 200-gm. sample was taken for chemical determination from each

[2] The chemical determinations were made by John B. Smith, chemist.

[3] Correspondence with H. G. M. Jacobson, Connecticut Agricultural Experiment Station.

pot. Previous to the planting of the lettuce 7.5 gm. $CaSO_4$, 1 gm. $Ca(NO_3)_2 \cdot 4H_2O$, and 0.5 gm. K_2SO_4 were mixed with the soil in each pot. Once during the growth of the lettuce, 1 gm. of $NaNO_3$ was applied to each pot in solution.

After the lettuce was harvested, the soil was removed from each pot and remixed. Then 1 gm. $Ca(NO_3)_2 \cdot 4H_2O$ and 0.5 gm. K_2SO_4 were mixed with the soil from each pot. During the growth of the barley 1 gm. $NaNO_3$ and 0.5 gm. K_2SO_4 were added. The barley seed was planted May 18, 1929, and

TABLE 4

Chemical determinations and dry-weight yields of barley from acid soils

LOCATION	SOIL TYPE	REACTION		JONES CaO REQUIREMENT FOR 2,000,000 POUNDS SOIL	ACTIVE Al_2O_3 IN DRY SOIL	P_2O_5 CONTENT OF BARLEY (DRY MATTER BASIS)	RELATIVE YIELD OF BARLEY
		pH	Wherry's scale active acidity				
				pounds	*p.p.m.*	*per cent*	
Group I:							
Indiana	Clermont silt loam	4.58	250	2,817	81	0.328	8.5
Rhode Island	Merrimac silt loam	4.71	200	2,907	840	0.636	100.0
Connecticut	Taugwank loam	4.75	160	3,645	905	0.324	16.0
New Jersey	Sassafras silt loam	4.83	160	2,502	494	0.464	10.2
Pennsylvania	De Kalb silt loam	4.90	125	2,646	428	0.133	6.8
Group II:							
Indiana	Scottsburg silt loam	5.04	100	4,509	67	0.405	144.0
South Carolina	Portsmouth sandy loam	5.07	80	3,168	117	0.498	176.2
Pennsylvania	Volusia silt loam	5.08	80	3,006	260	0.335	83.0
Connecticut	Hollis silt loam	5.18	63	2,925	519	0.409	132.0
Illinois	Muscatine silt loam	5.48	32	2,655	31	0.317	179.6
North Dakota	Fargo clay	5.76	16	2,250	Trace	0.483	137.3
Maine	Silt loam	5.83	16	1,512	589	0.344	13.5
Group III:							
Texas	Lufkin fine sandy loam	6.20	...	567	41	0.494	54.2
South Carolina	Greenville clay loam	7.01	...	261	79	0.552	81.3
Iowa	Carrington loam	7.25	...	270	18	0.509	120.3
Kentucky	Memphis silt loam	8.12	...	180	53	0.317	94.9

harvested when the heads were yellow on August 12, 1929. Dry-weight yields are shown in table 4.

It will be noted that no phosphoric acid was added to the soils during the growth experiment. This was because of the known effect (4) of phosphatic fertilizers in rendering aluminum non-toxic.

DISCUSSION

For ease in discussion of table 4, the data have been divided into three groups, arbitrarily, according to the reactions of the various soils. Group I

contains those soils with reactions below pH 5; group II is intermediate and less acid in reaction, containing those soils in the range of pH 5.04 to 5.83; whereas in group III are soils which are but slightly acid, having reactions above pH 6. Soils of group III will not be considered in this discussion. Relative barley yields are reported on the basis of Rhode Island station plat 82 as 100. The actual yield for this soil was 29.5 gm.

In order to obtain some measure of the possible effect of soil phosphorus on the growth of barley the crop was analyzed and the P_2O_5 reported on the dry-matter basis. It has been noted that where P_2O_5 is known to be high through fertilizer application it may be expected to show in increased amounts in the crop.

Group I

The dry-weight yields of barley from soils of group I are low, whereas the amounts of "active" aluminum present in dry soil and soluble in 0.5 *N* acetic acid are high. Exceptions to this statement are noted with barley on the Merrimac silt loam and with the aluminum in the case of the Clermont silt loam. The phosphoric acid content of the barley grown on this soil indicates that sufficient phosphoric acid may have been present, as a result of fertilization, so that to some extent the toxic effect of the aluminum was counteracted, and the growth of barley was inhibited to a lesser degree than on the other soils of this group.

The depression of barley on the Indiana soil may have been due to other inhibiting factors than aluminum or acidity. This soil described by Conner et al (5) as "a white, flat, timber soil, very low in organic matter and nitrogen." In addition to these deficiencies the tilth of this soil was found to be poor and the soil was inclined to puddle when an attempt was made to leach it.

If the Clermont silt loam soil is disregarded, the four soils remaining in group I indicate that either the aluminum or the reaction acidity was an inhibiting factor in the growth of barley, as both seem to correlate with the low relative dry-weight yields.

Group II

Since the reaction range of soils in Group II is between pH 5.04 and 5.83 and the dry-weight yields of barley vary from 13.5 to 179.6 gm. and are not consistent in their variation, no correlation seems to exist with reaction acidity. On the other hand if each soil be considered separately and the relative yield checked with the amount of "active" aluminum in the soil, in all cases, except that of the Hollis silt loam, where the aluminum is high, the relative dry weight is low and *vice versa.* The only reason for this discrepancy with the Connecticut soil may be found in the somewhat higher content of phosphoric acid in the barley, which may reflect a tying-up of aluminum in the soil. This, however, is only suggestive. With the Maine soil, even with a reaction which

can hardly be considered as very acid, sufficient aluminum seems to have been present to depress the barley very seriously.

It is thus necessary to turn to the intermediate group II for a suggestion as to the inhibiting factor. Group I, possibly because of the genesis of the soils in that group, which Burgess offers as a reason for the correlation which he found between acidity and aluminum content, does not allow the separation of the factors depressing the barley crop. Group II, however, where the acidity range is much less and where a greater variation in soil genesis is found as a result of the wider geographical spread in locations from which the samples were obtained, points quite definitely to the "active" aluminum content as the limiting factor in the growth of barley.

SUMMARY

In this paper further evidence is offered indicating that in soil and solution cultures "active" aluminum has a greater inhibitory effect on the growth of lettuce and barley plants than has acidity.

Solution cultures of Cos lettuce seedlings grown in a pH range of 3.2 to 7.5 showed no appreciable differences in dry-weight yields although cultures to which aluminum sulfate had been added were much depressed in yield.

The dry-weight yields of barley plants grown in samples of acid soils from several different soil types and widely separated geographically, were more closely correlated with the "active" aluminum content of the soils than with acidity.

REFERENCES

(1) Burgess, Paul S. 1922 The reaction of soils in the field as influenced by the long-continued use of fertilizer chemicals. R. I. Agr. Exp. Sta. Bul. 189.

(2) Burgess, Paul S. 1923 A method for the determination of "active" aluminum in acid soils. *Soil Sci.* 15: 131–136.

(3) Burgess, Paul S. 1923. Comparison of "active" aluminum and hydrogen-ion concentration of widely separated acid soils. *Soil Sci.* 15: 407–412.

(4) Burgess, Paul S., and Pember, F. R. 1923 "Active" aluminum as a factor detrimental to crop production in many acid soils. R. I. Agr. Exp. Sta. Bul. 194.

(5) Conner, S. D., Walker, G. P., and Plice, M. J. 1927 Soil acidity and base exchange studies on two very acid soils. *Proc. and Papers First Internatl. Cong. Soil Sci.* 2: 1–8.

(6) Line, J. 1926 Aluminum and acid soils. *Jour. Agr. Sci.* 16: 335–364.

(7) Lundell, G. E. F., and Hoffman, J. I. 1924 The analysis of phosphate rock. *Jour. Assoc. Off. Agr. Chem.* 8: 184–206.

(8) McLean, Forman T., and Gilbert, Basil E. 1927 The relative aluminum tolerance of crop plants. *Soil Sci.* 24: 163–174.

(9) McLean, Forman T., and Gilbert, Basil E. 1928 Aluminum toxicity. *Plant Physiol.* 3: 293–302.

A SIMPLE METHOD OF ESTIMATING TOTAL EXCHANGEABLE BASES IN SOILS

AMAR NATH PURI

University Chemical Laboratory, Lahore, India

Received for publication June 16, 1930

Bases in soils exist in two forms: Free as carbonates, or combined with the colloidal complex. The latter are sometimes spoken of as "absorbtively bound" or exchangeable bases. They are determined by leaching the soil with a neutral salt solution like ammonium chloride (4), barium chloride (1), or sodium chloride (3); or with a dilute acid like 0.05 N HCl (2). Also by electrodialysis (5), or electrofiltration (6).

None of these methods, however, is free from objection, as a certain amount of free carbonates are bound to appear in the leachate; and the success of a method must inevitably rest on the prevention of carbonate dissolution. In Hissink's method (3), the soil is washed in two lots; the amount of calcium in the second lot is supposed to represent the quantity dissolved, and subtracting it from that in the first lot gives the exchangeable calcium. In the neutral salt displacement method, the soluble salts also appear with the exchangeable bases and introduce a corresponding error.

The evaluation of total exchangeable bases, would be of great importance in any system of genetic classification of soils. For routine work, however, a rapid and fairly accurate method that can be carried out with the minimum of equipment is needed. It is the object of this paper to outline such a method.

The method consists in the determination of carbonates (representing free bases), as well as total bases (free and exchangeable). The difference between the two values represents exchangeable bases.

DETERMINATION OF CARBONATES

Carbonates are determined by the titration method developed by the author and discussed elsewhere (7). The following is the outline of the method:

Ten grams of soil is stirred with 100 cc. of water in a conical flask and excess $CaSO_4$ (0.2 to 0.5 gm.) added. The mixture is brought to the boiling point over a sand bath or Rose burner. Then 10 cc. of 0.1 N Al_2Cl_6 solution is added and the suspension shaken a little. About 10 drops of Brom thymol blue indicator (1 per cent solution in 50 per cent alcohol) is added and the suspension allowed to settle after a vigorous shaking. The soil settles down in several minutes, when the color of the supernatant liquid is noted. Yellow indicates less than 1 per cent carbonates, green to blue indicates more than 1 per cent carbonates. Next, 10 drops of Brom cresol green indicator (1 per cent solution in 50 per cent alcohol) is added and the color again noted. Golden yellow indicates absence of carbonates, green indicates less than 1 per cent

carbonates, deep bluish green indicates more than 1 per cent carbonates. If carbonates are present the procedure is as follows: The flask is brought to the boiling point and the contents are titrated against 0.5 *N* H_2SO_4. The suspension is boiled for a minute or two after each addition and allowed to settle for two minutes. This however, is only necessary in the later stages of titration as it is easy to see the green edge in the beginning. *The titration is complete when the color of the supernatant liquid is golden yellow which persists after the suspension has been boiled for several minutes, and allowed to settle for a minute or two.* The amount of H_2SO_4 used is equivalent to the total carbonates, or free bases in the soil. As a rule 8 to 12 titrations are started at once and completed within several hours.

The method has been shown to give values agreeing with the standard method in which CO_2 is liberated by acid treatment and measured by absorption in baryta. For fuller details the original paper should be consulted.

DETERMINATION OF TOTAL BASES

To 10 gm. of soil contained in a 500-cc. reagent bottle, sufficient 0.5 *N* HCl is added to break up all the carbonates, as determined by the foregoing method. Then 100 cc. of 0.1 *N* HCl is added and the mixture left for a few hours being shaken frequently, till the evolution of CO_2 bubbles has subsided. The bottle is next tightly corked with a rubber bung and mechanically shaken for about two hours. The suspension is filtered through a Büchner funnel, preferably without suction. For this purpose it is necessary to stick the filter paper to the funnel by running a little molten wax around the edges or by applying collodion with a camel's-hair brush. In either case the filter paper should be moistened first, and held on the funnel by suction. The soil on the filter paper is washed with 400 cc. of 0.05 *N* HCl in 100-cc. lots, and then with 200 cc. of water in 100-cc. lots. The filtrate is then made up to a definite volume and an aliquot titrated against standard alkali, phenolphthalein being used as indicator. The decrease in the concentration of the acid used is equivalent to the total bases present.

The quantities of acid solution recommended for washing need not be rigidly adhered to, though they should never be exceeded. For light soils and those containing little or no carbonates, 200 cc. of 0.05 *N* HCl instead of 400 cc. is quite enough. The main point is to wash the soil with an excess of acid (not too strong), till the exchangeable calcium is removed, and determine the amount of acid thus neutralized. The treatment outlined was found to accomplish this even in soils containing more than 50 per cent clay.

DISCUSSION OF RESULTS

In table 1 are given total exchangeable bases for 49 soils determined by the method described. The pH values (by H electrode 1:5 soil-water ratio) and the clay contents of the soils are also recorded. For convenience of reference a column has been added giving the exchangeable bases per gram of clay; these values show a rough correlation with the pH values, and for soils containing an excess of carbonates they give some idea of the base exchange capacity of

TABLE 1

Total bases, carbonates, and exchangeable bases in various soils

SOIL NO. P.C.	CLAY	TOTAL BASES, M.E. PER GM. OF SOIL	CARBONATES, M.E. PER GM. OF SOIL	EXCHANGEABLE BASES, MILLI-EQUIVALENTS PER GM.		pH
				Soil	Clay	
	per cent					
1	11.3	6.964	6.85	0.114	1.01	8.45
2	59.3	1.818	1.00	0.818	1.38	8.21
3	62.2	0.750	0.10	0.650	1.04	7.64
4	15.2	0.366	0.20	0.166	1.09	8.55
5	12.3	0.512	0.374	0.138	1.12	8.77
6	28.4	0.085	Nil	0.085	0.299	5.29
7	21.8	1.468	1.30	0.168	0.77	9.58
8	25.2	0.281	0.15	0.131	0.52	8.41
9	21.6	0.1125	0.024	0.0885	0.41	5.76
10	35.6	0.584	0.225	0.359	1.01	8.71
11	32.8	0.849	0.50	0.349	1.06	8.77
12	7.2	0.075	Nil	0.075	1.04	5.84
13	58.9	0.979	0.30	0.679	1.16	8.53
14	21.5	0.019	Nil	0.019	0.088	5.37
15	22.4	0.125	0.05	0.075	0.33	7.71
16	8.7	0.755	0.374	0.381	4.38	8.74
17	14.1	0.275	0.10	0.175	1.24	8.20
18	22.6	0.083	0.024	0.059	0.26	5.79
19	42.3	0.691	0.20	0.491	1.16	8.40
20	8.1	0.019	0.024	Nil	Nil	5.64
21	13.5	0.834	0.574	0.26	1.93	8.25
22	15.1	0.158	0.024	0.134	0.89	6.85
23	11.3	0.157	0.05	0.107	0.95	7.41
24	9.7	0.598	0.35	0.248	2.56	8.59
25	4.0	0.038	Nil	0.038	0.95	7.40
26	22.5	0.078	Nil	0.078	0.35	8.11
27	53.2	1.508	0.724	0.774	1.45	9.03
28	44.6	0.648	0.174	0.474	1.06	8.38
29	63.0	0.638	0.074	0.564	0.90	8.05
30	54.1	1.398	0.824	0.574	1.06	8.45
31	22.8	0.94	0.804	0.136	0.60	8.01
32	64.6	0.24	0.150	0.09	0.14	5.05
33	2.6	1.175	0.95	0.225	0.87	10.18
34	11.3	0.190	Nil	0.190	1.68	7.63
35	18.3	0.095	0.025	0.07	0.38	7.98
36	11.7	0.18	0.05	0.13	1.11	8.40
37	4.1	0.05	0.025	0.025	0.61	5.72
38	52.9	0.59	0.10	0.49	0.93	8.29
39	8.4	0.51	0.20	0.31	3.69	9.11
40	13.1	0.17	0.024	0.146	1.11	7.65
41	53.4	2.34	1.75	0.59	1.10	8.74
42	53.4	1.905	1.15	0.755	1.41	9.00
43	21.6	1.39	1.124	0.266	1.23	8.41
44	8.4	1.62	1.374	0.246	2.93	8.54
45	11.1	0.09	0.05	0.40	0.36	7.45
46	56.4	0.66	0.10	0.56	0.99	7.63
47	17.1	1.44	1.15	0.29	1.70	9.98
48	19.8	1.41	1.25	0.16	0.81	8.55
49	23.3	0.095	0.05	0.045	0.19	6.33

colloids, for such soils may be considered as saturated with bases in the sense that any excess added would be converted into carbonates.

That the determination of exchangeable bases by neutral salt reaction can be very misleading, is shown by the following experiment in which a heavy black cotton soil (P.C.2) was leached with *N* and 0.2 *N* NH_4Cl, and with 0.1 *N* $BaCl_2$ solution, in 100-cc. lots. Ca was determined in 400-cc. lots of the leachate. The results are recorded in table 2.

Table 2 shows that NH_4Cl solution is not a suitable reagent for determining exchangeable bases; $BaCl_2$ solution on account of the limited solubility of $CaCO_3$ in it seems to be much superior (1). Considering that leaching with $BaCl_2$ solution was not carried beyond 1,200 cc., and bearing in mind that only Ca was determined in the leachate, the auther's method may be taken as comparable to $BaCl_2$ washing.

TABLE 2

Removal of Ca from soil P.C.2 in successive washings with NH_4Cl and $BaCl_2$ solutions

SUCCESSIVE 400-CC. WASHINGS	M.E. OF Ca FROM 10 GM. OF SOIL			TOTAL EXCHANGEABLE BASES BY THE AUTHOR'S METHOD, M.E. PER 10 GM. OF SOIL
	N NH_4Cl	0.2 *N* NH_4Cl	0.1 *N* $BaCl_2$	
1	8.8	7.0	5.6	....
2	2.825	2.84	0.38	....
3	1.11	1.6	0.48	....
4	1.96	0.95	....	....
5	1.21	0.91	....	....
6	0.165	....	....	....
Total.........	16.07	13.30	6.44	8.18

SUMMARY

A method for the estimation of total exchangeable bases in soils is outlined. It consists in the determination of carbonates by the author's titration method, and of total bases by dilute HCl treatment; the difference between the two values giving the exchangeable bases.

The results of such determinations on 49 soils from different parts of India are recorded.

REFERENCES

(1) BURGESS, P. S., AND BREAZEALE, J. F. 1926 Methods for determining the replaceable bases of soils, either in the presence or absence of alkali salts. Ariz. Agr. Exp. Sta. Tech. Bul. 9.

(2) GEDROIZ, K. K. 1923 The hydrochloric acid method for determining in the soil the cations present in an absorbed condition. *Soil. Sci.* 16: 473–474.

(3) HISSINK, D. J. 1927 Methods for the determination of exchangeable bases. *Trans. Int. Soc. Soil Sci. (Groningen) II Commission*, Vol. B: 181.

(4) KELLY, W. P., AND BROWN, S. M. 1924 Replaceable bases in soils. Univ. Calif. Pubs. Tech. Paper 15.

(5) MATTSON, SANTE 1928 Electrodialysis of the colloidal soil material and the exchangeable bases. *Jour. Agr. Res.* 33: 553–567.

(6) PURI, A. N. 1930 Electrofiltration a new method of removing exchangeable bases from soil colloids. *Soil. Sci.* 30: 413–419.

(7) PURI, A. N. 1930 A new method of estimating total carbonates in soils. Agr. Res. Inst. Pusa Bul. 206.

SAMPLING MARKET-GARDEN SOILS FOR NITRATES[1]

J. ERIC BLANEY AND JOHN B. SMITH

Rhode Island Agricultural Experiment Station

Received for publication July 16, 1930

In anticipation of a growing need for knowledge regarding methods for chemical control of available soil nitrogen during the growth of crops, especially of market-garden vegetables, a study has been made of the distribution of soil nitrates under the heterogeneity that exists in such conditions, with a view to deciding upon a sampling scheme. In a strict sense the data have only a local application, but publication of the results supplements the findings of Waynick (4) and Prince (3) who studied more uniform conditions, and may aid those who wish to work with drilled vegetable crops growing in highly-manured soils, fertilized with hand-distributed chemicals, and further supplemented from time to time with side-dressings of soluble nitrogen.

EXPERIMENTAL PROCEDURE

Soil

The areas selected were three $\frac{1}{80}$-acre plats of a market-garden rotation (*W*) last described by Hartwell and Crandall (2). The soil has been classified as Merrimac silt loam. Since 1916, these plats have received considerable quantities of stable manure, for which a mixture of manure with vegetable compost has been substituted recently. The manure applied in the spring has been supplemented liberally by complete inorganic fertilizers, both in the spring and before the second crops that are planted at midseason. Sufficient lime has been used to maintain a neutral reaction. The initial application of fertilizer for the crops studied was sown broadcast by hand, but certain of the early crops that preceded these were fertilized in the hill, and all crops have had side-dressings of soluble nitrogen at various times in the growing season. Thus, the original uniformity of the soil has been disturbed by the unevenness unavoidable in distributing fertilizer by hand, hill fertilization in a few instances, the application of soluble nitrogen between the rows of the growing crops, and variable absorption by the crops.

[1] Published by permission of the director of the Agricultural Experiment Station as Contribution No. 399.

Sampling and analysis

The samples were taken during September and October, 1928, after the harvest of the early crops and while beets, celery, and spinach were growing. Eight borings were taken from each of five rows extending lengthwise of a rectangular plat, and within 6 inches of the crop row. The rows selected were approximately 4 feet apart and the borings were spaced 5 feet apart in the row. The borings are not numbered in the tables presented, but the location of each individual result in the table corresponds to its location on the rectangular plat. Thus, the tables represent sampling diagrams as well as records of the data.

Each sample consisted of a single core, 0.75 inches in diameter, taken with a "trier" to a depth of 7 inches. The samples were brought to the laboratory, passed through a 5-mesh sieve, and portions for analysis were weighed immediately. Nitrate nitrogen was determined by the phenoldisulfonic acid method as modified by Harper (1). All results were corrected to a dry soil basis by using a uniform correction factor for each set of borings. The factor was calculated from the mean of the moisture determinations for 10 cores well distributed over the area. Preliminary moisture determinations in a larger number of borings showed small variations and it was calculated that the probable error of the mean of 10 determinations was less than 0.5 per cent of that mean.

The accuracy of the laboratory method for nitrate nitrogen was checked by determinations in 28 duplicate portions of soil. The greatest variation noted was 1 p.p.m. of nitrate nitrogen in dry soil. As this is the smallest difference that could be shown between two colorimeter readings in the dilutions of extract that were used, the actual variations were less than this value. It is recognized that there is a laboratory error that varies in magnitude with the concentration of nitrates in the solution analyzed, but it is small in comparison with the sampling error and has been disregarded.

Statistical methods[2]

The customary formulas for standard deviation (σ) and probable error of the mean (PE_m) were used.

$$PE_m = \frac{0.6745\ \sigma}{\sqrt{N}}$$

In the formula for σ, $N-1$ was used in the denominator rather than N for greater accuracy in dealing with small groups of data. The application of these formulas to soil-nitrate studies has been discussed fully by Waynick (4) and Prince (3). For comparison of the nitrate uniformity under different soil and

[2] The authors are indebted to Dr. T. E. Odland for suggestions as to the use of statistical formulas.

crop conditions the errors have been calculated as the percentage ratios of the errors to their means $\left(\frac{100\ PE_m}{M}\right)$. Thus, for a soil containing 20 p.p.m. of nitrate nitrogen, a PE_m of 5 per cent signifies that the chances are even that the true mean lies between 20 $\pm$ 1 p.p.m., and about 30 to 1 that it lies within 3 PE_m, or between 17 and 23 p.p.m. in this instance.

The statistical methods employed are applicable only to data that are distributed uniformly about the mean. Although this uniformity can usually be assumed, it seemed desirable to test the assumption in this instance. For this purpose nitrate nitrogen was determined in 95 borings taken from a $\frac{1}{80}$-acre plat. The results were grouped in classes with limits 5 p.p.m. apart (table 3). Although a line connecting the midpoints of the upper sides of the rectangles of the histogram charted from these data was somewhat asymmetrical, the mean fell very close to the midpoint of the modal class, and it seems reasonable to assume that the lack of symmetry is caused by a dearth of data rather than by a true lack of uniformity in distribution. The evidence of normal distribution of results about the mean was accepted as satisfactory.

To show the reliability of composites of different numbers of borings, the constants have been calculated for various combinations of the individual results as well as for the whole number taken from each plat. Each group of 20, 13, and 6 results is made up from borings spaced as uniformly as possible over the plat and no result was used twice in groups of the same size.

At the outset, a probable error of 5 per cent of the mean was chosen as the maximum allowable variation and the data have been studied with this in mind.

DISCUSSION OF RESULTS

The data reported in tables 1, 2, and 3 were obtained from a plat planted to spinach September 5. The early tomatoes that preceded the spinach had received 60 pounds[3] of nitrogen applied close to the plants. A mixture of nitrate of soda and sulfate of ammonia sufficient to supply 20 pounds of nitrogen was broadcast and harrowed in when the spinach was planted, followed by a side-dressing of 30 pounds on October 4. At the first sampling date, September 18, the plants were still in the cotyledon stage and could not have affected samples taken midway between the rows. This set of results has been labelled "fallow" (table 1). The second set of samples was taken a month later, 2 weeks after the application of the side-dressing, and when the crop was one-quarter grown. The borings were taken within 6 inches of the row (table 2). A third set, containing 95 borings, was taken October 23 after the plants were more than half grown. To save space, only a statistical summary of the 95 borings is recorded in table 3. The data were grouped in classes for the construction of the distribution diagram already mentioned,

[3] All applications are reported on the acre basis.

but the statistical constants were calculated from the individual determinations.

The quantities of nitrate nitrogen were within the normal range and decreased from 41.7 p.p.m. in the fallow to 35.7 p.p.m., when the crop was quarter grown, and to 13 p.p.m. at the last sampling date. The probable errors calculated as percentages of the means based on approximately 40 borings increased for the last sampling as compared with the first two, but the general uniformity was greater than for the other crops studied. There was a decided increase in the probable error for composites of less than 40 borings, and the error was usually greater than 5 per cent when less than 20 borings were considered. There is a noticeable lack of uniformity among the different groups of less than 20 borings.

TABLE 1

Nitrate nitrogen in fallow, September 18, 1928—Plat 115

Individual borings, p.p.m. of NO_3-N in dry soil

25	25	25	35	35	29	51	58
58	35	19	58	41	82	19	62
37	..	33	45	62	58	53	41
53	49	33	33	33	26	27	41
37	29	41	58	49	41	33	58

Statistical summary

39 BORINGS		20 BORINGS*		13 BORINGS*		6 BORINGS*	
M PE_m	*per cent*	M PE_m	*per cent*	M PE_m	*per cent*	M PE_m	*per cent*
41.7 ±1.5	4	38.2 ±1.9	5	42.5 ±1.8	4	42.0 ±2.6	6
....	..	†45.4 ±2.3	5	42.0 ±3.4	8	49.5 ±5.9	12
....	..	....	..	40.7 ±2.8	7	37.0 ±4.7	13
....	..	....	..	....	..	43.2 ±3.2	7
....	..	....	..	....	..	36.2 ±3.7	10
....	..	....	..	....	..	41.5 ±3.7	9

* The results are combined so that each group of borings is distributed over the area with equal uniformity.

† Nineteen borings.

Two sets of samples were taken under beets; the first on September 5, five weeks after the crop was planted, and the second October 9, when the plants were full grown. Liberal quantities of nitrogen were applied broadcast and in side-dressings for the cabbage that preceded the beets. The data are reported in tables 4 and 5. Seventy-five pounds of nitrogen in nitrate of soda and sulfate of ammonia were applied in three equal applications, the first as a broadcast at planting time, the second and third as side-dressings on September 21 and October 3. The plat is manured twice in each 3-year period but not in the cabbage-beet year.

TABLE 2

Nitrate nitrogen under small spinach, October 18, 1928—Plat 115

Individual borings, p.p.m. of NO_3-N in dry soil

27	33	23	71	39	31	31	33
31	27	33	23	19	39	37	41
27	21	17	27	27	50	35	35
44	46	54	50	56	33	37	37
37	29	33	48	37	33	31	46

Statistical summary

40 BORINGS		20 BORINGS*		13 BORINGS*		6 BORINGS*	
$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*
35.7 ±1.2	3	34.0 ±1.5	4	36.9 ±2.7	7	29.2 ±2.9	10
....	..	38.0 ±1.8	5	36.2 ±1.5	4	35.8 ±1.8	5
....	..	....	..	33.2 ±1.8	5	34.8 ±3.0	9
....	..	....	..	....	..	44.7 ±4.5	11
....	..	....	..	....	..	37.2 ±2.8	8
....	..	....	..	....	..	31.8 ±2.7	8

* The results are combined so that each group of borings is distributed over the area with equal uniformity.

TABLE 3

Nitrate nitrogen under half-grown spinach, October 23, 1928—Plat 115, 95 borings

NO_3-N, p.p.m.	0–5	5. 1–10	10. 1–15	15. 1–20	20. 1–25	25. 1–30	30. 1–35	35. 1–40
Number of borings.......	13	24	29	19	5	2	2	1

Statistical summary

Calculated from individual determinations of NO_3-N in p.p.m

95 BORINGS*		48 BORINGS*		19 BORINGS*		12 BORINGS*		6 BORINGS*	
$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*
13.0 ±0.5	4	12.5 ±0.7	6	14.0 ±1.0	7	12.1 ±1.3	11	13.2 ±0.8	6
....	..	13.5 ±0.6	4	11.6 ±1.0	9	14.9 ±1.5	10	9.8 ±2.1	21
....	..	....	..	13.6 ±0.8	6	13.8 ±1.6	12	13.2 ±1.6	12
....	..	....	..	14.9 ±1.4	9	12.2 ±0.9	7	12.2 ±1.1	9
....	..	....	..	11.7 ±1.2	10	12.1 ±1.5	12	10.2 ±0.8	8
....	..	....	..	....	..	15.7 ±1.7	11	15.7 ±2.7	17
....	..	....	..	....	..	12.1 ±1.0	8	13.3 ±2.9	22
....	..	....	..	....	..	....	..	10.7 ±1.5	14
....	..	....	..	....	..	....	..	10.3 ±1.5	15
....	..	....	..	....	..	....	..	17.2 ±3.2	19

* The results are combined so that each group of borings is distributed over the area with equal uniformity.

On September 5 there were 29.9 p.p.m. of nitrate nitrogen under the half-grown beets but despite the two side-dressings, the concentration was reduced to 4 p.p.m. by October 9 when the crop was ready for harvest. The 4 p.p.m.

TABLE 4

Nitrate nitrogen under half-grown beets, September 5, 1928—Plat 112

Individual borings, p.p.m. of NO_3-N in dry soil

28	30	24	30	32	22	24	51
67	36	22	36	42	28	63	71
32	26	32	30	..	34	32	34
42	32	32	32	32	34	47	38
4	7	6	6	3	10	10	6

Statistical summary

39 borings		20 borings*		13 borings*		6 borings*	
M PE$_m$	*per cent*	*M PE$_m$*	*per cent*	*M PE$_m$*	*per cent*	*M PE$_m$*	*per cent*
29.9 ±1.8	6	†30.2 ±2.7	9	31.8 ±2.9	9	32.7 ±1.8	6
....	..	30.0 ±2.4	8	26.5 ±2.4	9	29.2 ±4.1	14
....	..	....	..	32.0 ±3.8	10	37.8 ±6.4	17
....	..	....	..	....	..	34.5 ±5.8	17
....	..	....	..	....	..	26.7 ±3.1	12
....	..	....	..	....	..	29.3 ±4.4	15

* The results are combined so that each group of borings is distributed over the area with equal uniformity.

† Nineteen borings.

TABLE 5

Nitrate nitrogen under full-grown beets, October 9, 1928—Plat 112

Individual borings, p.p.m. of NO_3-N in dry soil

2.8	2.8	2.6	2.4	3.8	4.8	5.1	9.2
2.6	2.6	2.8	7.0	3.2	3.6	7.4	4.6
3.8	3.4	2.8	7.6	7.2	8.8	5.0	6.6
4.6	1.6	1.2	12.0	1.6	2.0	3.8	1.4
2.4	1.2	1.6	1.4	2.4	2.4	7.0	4.6

Mean of 40 borings 4.09±0.28

$$\frac{PE_m \times 100}{M} = 7 \text{ per cent}$$

level was so low that the accuracy of the laboratory method scarcely justifies calculation of the statistical constants. By extending the calculations somewhat beyond the exact accuracy of the determinations, however, a value for the probable error was found and is reported in table 5. No great reliance

should be placed in this constant, but it is evident that the variation is high. The probable error is also high for the half-grown beets, 6 per cent, where 39 borings were taken, and much greater for composites of lesser numbers of borings. The nitrogen in the borings from just inside the outer row of beets represented by the lower line of results in table 4, is much less than that from the remainder of the plat, demonstrating a lack of uniformity in fertilizer distribution.

Samples were taken under half-grown celery on September 14 (table 6). This crop was preceded by lettuce. A heavy application of manure compost was used in the spring and liberal quantities of fertilizer nitrogen were broadcast for the lettuce. Celery plants were set in July but no nitrogen or potash

TABLE 6

Nitrate nitrogen under half-grown celery, September 14, 1928—Plat 118

Individual borings, p.p.m. of NO_3-N in dry soil

16	27	37	41	29	16	16	62
23	12	12	8	41	51	41	25
47	33	12	51	31	23	25	10
7	14	3	9	6	18	6	16
10	25	18	8	8	33	41	70

Statistical summary

40 BORINGS		20 BORINGS*		13 BORINGS*		6 BORINGS*	
$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*	$M\ PE_m$	*per cent*
24.5 ±1.7	7	21.5 ±2.1	10	18.5 ±2.2	12	16.3 ±3.5	21
....	..	27.6 ±2.8	10	30.1 ±3.3	11	36.8 ±5.6	15
....	..	....	..	21.5 ±2.6	12	24.2 ±4.2	17
....	..	....	..	....	..	22.5 ±3.1	14
....	..	....	..	....	..	22.8 ±4.0	18
....	..	....	..	....	..	15.5 ±2.6	17

* The results are combined so that each group of borings is distributed over the area with equal uniformity.

was used until August 10 to avoid high concentrations of soluble salts about the tender plants in rather dry soil. On August 10, 25 pounds of nitrogen in a mixture of nitrate of soda and sulfate of ammonia was applied as a side-dressing and again on August 31. The plants available on July 12 were only sufficient to set five rows and the remaining two were filled in July 25. Nitrate determinations from borings beside the two rows of younger plants are reported in the top line of table 6. The shorter feeding time was reflected in a slightly higher average for nitrate nitrogen than that for the remainder of the data. A greater variation is evidenced, however, in the fourth line of results, which are less than half the average for borings in the other rows. This discrepancy is ascribed to uneven distribution of the side dressings.

This set of results represents the extreme variability found for this type of cropping, and the 40 borings taken were not sufficient to reduce the error to 5 per cent. Composites of 20 borings or less gave errors of 10 per cent or greater.

A summary of the data (table 7) for the six different soil and crop conditions shows the greatest uniformity for the fallow and for small spinach. As the crops increased in size the errors were larger. For the most favorable condition studied the probable error varied from 3 per cent with 40 borings to 7 per cent as an average for 6 groups of 6 borings. For the least favorable, the probable errors increased from 7 per cent for 40 borings to 17 per cent for an average of 6 groups of 6 borings. At the best these errors are large, but the increased accuracy from larger numbers of borings is clear.

Apparently for conditions similar to those studied composite samples of 40 borings from $\frac{1}{80}$-acre areas of drilled market-garden crops may be expected

TABLE 7

Summary of probable errors for nitrate nitrogen under growing crops, expressed as the percentage ratios of the probable errors to the respective means of different combinations of data from individual borings

$\frac{1}{80}$ ACRE	BORINGS	PE_m	BORINGS	PE_m*	BORINGS	PE_m†	BORINGS	PE_m‡
	number	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
Fallow	39	4	20	5	13	6	6	10
Small spinach	40	3	20	5	13	5	6	7
Half-grown spinach	48	5	19	8	12	10	6	14
Half-grown beets	39	6	20	9	13	9	6	14
Full-grown beets	40	7	..	..	..	..	..	..
Half-grown celery	40	7	20	10	13	12	6	17

* Average of two groups of borings.
† Average of three groups of borings.
‡ Average of six groups of borings.

to give errors of approximately 5 per cent; 20 borings, 7 per cent; 13 borings, 8 per cent, and 6 borings, 12 per cent.

COMPARISON OF NITRATE SAMPLING DATA FOR DIFFERENT CROPPING CONDITIONS AND GEOGRAPHICAL LOCATIONS

The data of Waynick (4) for a California fallow, presumably unfertilized and of Prince (3) for timothy sod in New Jersey were compared with the Rhode Island results under vegetables by segregating the data for $\frac{1}{80}$-acre areas measured to scale on the published diagrams of the sampling schemes. The comparison is not strictly accurate since Waynick took borings that were 6 inches in diameter; Prince used composites of three 1-inch borings taken close together to form units; whereas the results in this paper are based on individual cores $\frac{3}{4}$ inch in diameter. The depth was approximately 7 inches in each

case. The larger borings would undoubtedly tend to overcome the lack of uniformity.

The standard deviation (σ) was determined for the greatest number of borings reported for the $\frac{1}{80}$-acre area selected in each case. By substituting this value in the formula for PE_m any number of samples (N) may be assumed and the corresponding theoretical probable error (PE_m) calculated; or a desired PE_m may be substituted in the equation and the number of samples required to give this error may be determined.

TABLE 8

Comparison of the uniformity of soils from widely separated areas, with respect to nitrate nitrogen and the number of borings necessary for an adequate sample

Results are calculated to the basis of $\frac{1}{80}$-acre and probable errors are expressed as per cent of the mean

	NUMBER OF BORINGS USED AS BASIS OF CALCULATION	APPROXIMATE PE_m TO BE EXPECTED FROM COMPOSITE SAMPLES				NUMBER OF BORINGS REQUIRED TO GIVE PROBABLE ERROR OF	
		40 borings	20 borings	13 borings	6 borings	3	5
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Rhode Island:							
Fallow	39	4	5	6	9	59	21
Small spinach	40	3	4	6	8	48	17
Half-grown spinach	48	5	8	10	14	135	49
Half-grown beets	39	6	8	10	15	151	55
Half-grown celery	40	7	10	13	18	225	81
California:*							
Fallow	33	3	4	5	7	34	12
New Jersey:†							
Timothy (manured)	16	3	5	6	9	53	19
Timothy (not fertilized)	16	2	3	4	5	18	7
Timothy ($NaNO_3$)	16	2	3	3	5	18	6

* Calculated from data published by Waynick (4).
† Calculated from data published by Prince (3).

By the use of this method the errors to be expected for nitrate nitrogen in composites of 40, 20, 13, and 6 borings have been calculated for the various soil and crop conditions (table 8). For the larger groups, the values found compare very closely with those obtained by the actual grouping of individual results (table 7), and for the average errors of several smaller combinations of data, but the errors from single composites of 13 or of 6 individuals often vary widely from the average. Great reliance cannot be placed in errors from composites made up of small groups of individuals when these are calculated from the standard deviations of larger groups from similar conditions.

The numbers of borings necessary to limit the errors to 3 per cent and to 5 per cent were calculated and are also presented in table 8.

The most uniform conditions were the unmanured New Jersey plats under timothy, followed by the California fallow and the New Jersey plat with manure. The Rhode Island areas were the least uniform, as would be expected, because of the heavy annual applications of manure compost, hand distribution of large mineral fertilizer supplements, soluble nitrogen top-dressings, and the nitrate removal by growing vegetable crops in drills during the sampling period. Among the different Rhode Island conditions the best uniformity was for a fallow and for small spinach. As the crops became larger the error increased but differences noted among the different crops, spinach beets, and celery, were not sufficient to be ascribed to the specific crops.

The numbers of borings required to give probable errors of 3 per cent for $\frac{1}{80}$-acre areas varied from 18 borings for the unmanured New Jersey plats under the timothy to 225 borings under growing celery in Rhode Island. For 5 per cent errors the corresponding numbers of borings were 6 and 81.

It is evident that each problem should be studied before deciding upon the sampling method, but certain general conditions may be grouped. Apparently fallows and broadcast crops will give probable errors of less than 3 per cent for nitrate nitrogen if 40 borings are taken for $\frac{1}{80}$-acre areas and less than 5 per cent when 25 borings are used. Drilled crops in manured soils supplemented by applications of chemical fertilizer require a minimum of 50 borings to reduce the probable error to approximately 5 per cent, and especially unfavorable conditions may require greater numbers.

SUMMARY

Nitrate nitrogen was determined in 40 cores of soil from manured, limed, and fertilized soils under drilled market-garden crops. Sets of samples were taken from fallow and from under small spinach, half-grown spinach, half-grown and full-grown beets, and half-grown celery. The uniformity of nitrate distribution under these conditions, as shown by the usual statistical constants, was markedly less than that noted by other workers for fallow and for timothy sod. A minimum of 50 borings proved necessary for $\frac{1}{80}$-acre areas of drilled vegetable crops to reduce the probable error for nitrate nitrogen to approximately 5 per cent of the mean.

REFERENCES

(1) HARPER, H. J. 1924 The accurate determination of nitrates in soil. *Jour. Indus. and Engin. Chem.* 16: 180–183.

(2) HARTWELL, BURT L., AND CRANDALL, F. K. 1928 The substitution of stable manure by fertilizers, green manures, and peat. III. R. I. Agr. Exp. Sta. Bul. 216.

(3) PRINCE, ARTHUR L. 1923 Variability of nitrates and total nitrogen in soils. *Soil Sci.* 15: 395–405.

(4) WAYNICK, DEAN DAVID 1918 Variability in soils and its significance to past and future soil investigations. I. A statistical study of nitrification in soil. *Univ. Calif. Pub. Agr. Sci.* 3: 243–270.

THE CONCEPTION OF FLOW-PLASTICITY AS APPLIED TO SOILS

G. W. SCOTT BLAIR[1]

Cornell University

Received for publication July 5, 1930

When a paste of soil[2] or clay in water is caused to flow through a capillary tube at a series of different pressures, the volume of the flow during each second being measured at each pressure, a flow-curve may be obtained by plotting pressure (P) against flow (V), which, it has been shown (5), may be divided into four distinct regions. In the first region of pressure, there is no flow at all, the limiting pressure for this region being a sort of adhesion value, or "yield-value" (1) for the water layer separating the body of paste in the tube from the glass wall. Let this limiting pressure be disignated a.

As soon as a is reached, the body of paste starts to flow as a solid block or plug along the tube, sliding through the water envelope, which remains constant in thickness. This second region of the flow-curve is linear, the straight line hitting the pressure axis sharply at a.

When a second critical pressure, b, is reached, a telescopic type of flow starts at the wall: and as pressure is still further increased, the flow-curve slopes very steeply upwards, as the width of the region of telescopic (or "fluid") flow increases in thickness toward the centre of the tube at the expense of the central plug.

Finally, in the fourth region, the "plug" has virtually disappeared, and the material streams as a fluid, giving a straight-line flow-curve having a slope about a hundred times that of the second region, or plug straight line.

The critical pressures a and b depend on the radius and length of the capillary tube used, but it has been shown that if we set

$$A = \frac{aR}{2L} \quad \text{and} \quad B = \frac{bR}{2L}$$

---

[1] The author, who is a staff member of the Rothamsted Experimental Station, Harpenden, England, performed the work reported in this paper, in the Baker Laboratory of Cornell University.

The author wishes to express his thanks to Prof. W. D. Bancroft of Cornell University for much help and many suggestions, to the director and staff of the New Jersey State Agricultural Experiment Station, and especially to Drs. J. S. Joffe and L. L. Lee, who supplied a number of soil samples and explained their classification.

[2] Gravel and coarse sand are first removed by sieving, the finest sieve used for the paste being 100 meshes to the inch.

where R is the radius, and L, the length of the tube, A and B, (which are now stresses per unit area on the wall of the tube), are independent of the values of R and L, provided that R and L are reasonable in value. The physics of the flow-curve in general is complex, and as this matter has already been discussed in detail, the discussion need not be repeated here (2, 3, 10).

For some time it was believed that the measurement of the slope and extrapolated intercept of the fourth region straight line [which were called "mobility" and "yield-value" respectively (2)], constituted a measurement of the "plasticity" of a material, though no exact definition was attached to that term. However, the term "plasticity" is a very ancient one; not only had "πλαστικός" a very definite meaning to the Greeks, but it is found that the same meaning, only rather more defined, is associated with the term by prac-

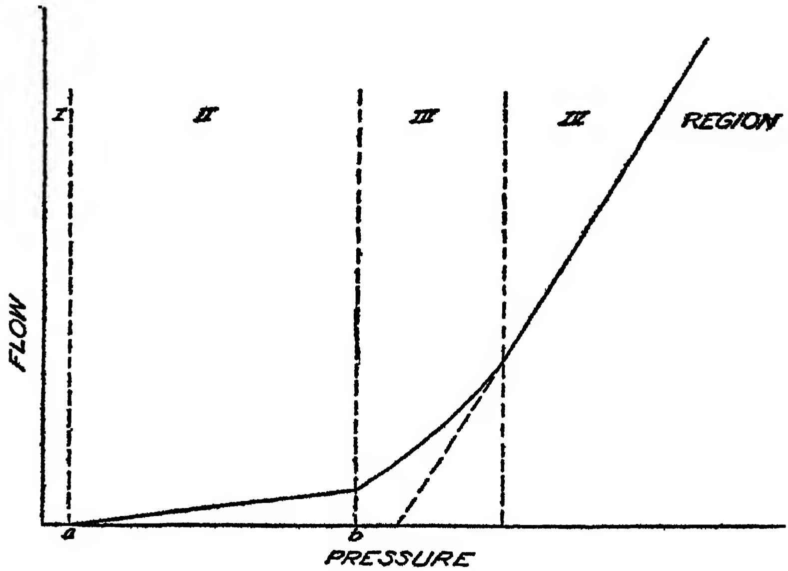


Fig. 1. Flow Curve of Soil

tical ceramists and others today. In a recent paper (4), this whole question has been discussed, and it was decided by the author that the following definition, given by Wilson (11), expressed, perhaps better than any other yet put forward, the idea underlying the term "plasticity," both with respect to its traditional and its modern requirements. Wilson definds plasticity as "that property which enables a material to be deformed continuously and permanently without rupture, during the application of a force which exceeds the yield-value of the material."

Now, it has been becoming increasingly clear for some time that plasticity defined in this way is *not* measured by mobility and yield-value; and yet it was felt that there should be some connection between plasticity and the flow-curve. In seeking for this connection, the following empirical fact was estab-

lished (4): *If pastes of clays in water are compared at such (different) concentrations of water that they show the same A-value, then the B-values measured at these concentrations are very closely related to the plasticity of the material, in the Wilson sense.* Since the test is still not fully understood, and since the use of the Wilson definition is, in a sense, arbitrary, it was decided to call this property "*flow-plasticity*," rather than simply "plasticity."

For convenience, the flow-plasticity is defined as the value of B in dynes per square millimetres, for a paste at such a water-content that it gives an A-value of 1 dyne/mm.^{2} In certain cases, however, 1 dyne/mm.^{2} is too high a value for A, for convenience and accuracy. For this reason, whereas B' will be written for the true flow-plasticity, B'_m will refer to a similar property, when A is m, instead of 1 dyne/mm.^{2}

In comparing two materials, the relative true flow-plasticities and the relative flow-plasticities at some value of A other than 1 should, of course, agree. This is not accurately the case: plasticity depends on the whole curve relating A to B for different concentrations, but the discrepancies are not great, and it is believed that, provided reasonable A-values[3] are chosen, the correlation with true plasticity is sufficiently close to be of considerable practical use.

The foregoing is a very brief description of the measuring and origin of flow-plasticity. For details, both in the theory and technique, the original papers (4, 5, 9, 10) should be referred to; but the discovery of a new method of measuring quickly, and with fair accuracy, something very closely allied to plasticity seemed of such significance with reference to soil work, that certain preliminary experiments were immediately undertaken. An outline of these experiments forms the subject of the present paper.

EFFECT OF LIME AND CHALK ON THE FLOW-PLASTICITY OF A HEAVY SOIL

It has already been shown (9) that if pastes of soils treated with chalk and lime are compared at the same moisture-content, the yield-values[4] are reduced, as compared with a control sample, to an extent depending on the time, and on the extent of the dressing. This reduction was shown to parallel a reduction in the force required to draw the plough through the soil. It is known that the addition of large dressings of chalk and lime make ploughing lighter; and since the less plastic the soil, the less the force required to deform the sod before its rupture by the mouldboard, and hence the less the energy wasted, it was thought that lime and chalk would be expected to reduce the flow-plasticity of a soil also. To test this prediction, a sample of Cornell University farm

[3] In actual practice, it is not necessary to adjust the A-value to exactly 1 dyne/mm.^{2} If a reading is taken at a value just greater than 1, and the material is then diluted and another reading is taken at a value rather less than 1, the B-value corresponding to $A = 1$ can be obtained from the other figures by means of a simple interpolation, since, over small ranges at least, A and B are linearly related.

[4] Intercept of the fourth region of the flow-curve extrapolated to the stress or pressure axis. Also called "rigidity" (or more correctly, "limit of rigidity") or "shearing strength."

soil (Dunkirk silty clay loam), taken from a plot which has been fallow for some years, and practically free of carbonates, was divided into a number of portions, and these were treated at the ordinary field condition of moisture with different amounts of chalk and lime.[5] Tests on flow-plasticity were made after 1–2 and 12–14 days respectively, and it was established that:

Lime, in quantities as small as 1 part to 1,000 reduces the plasticity of the soil.
This effect is more marked when the lime is finely ground than when it is coarsely ground.
Chalk reduces the plasticity, but to a lesser extent than does lime.
The reduction in plasticity is a slow process, being greater after a fortnight than after two days.

The results are given in table 1.

TABLE 1
*Results on tests of flow-plasticity**

TREATMENT	B′ (1–2 DAYS)	B′ (12–14 DAYS)
	$d/mm.^2$	d/mm^2
Control	10.6	10.6
1 part lime per 1,000	10.4	9.7
5 parts lime per 1,000	8.1	7.9
20 parts lime per 1,000	6.8	(8.8)†
20 parts lime per 1,000, but lime coarser ground	8.7	7.2
20 parts chalk per 1,000	8.0	7.8

* All flow-plasticity data given in this paper were obtained at a temperature of 25°C.
† This figure is unreliable, as there was a leak in the apparatus.

FLOW-PLASTICITY AS APPLIED TO THE STUDY OF THE SOIL-PROFILE

It is clear that, as the finer soil particles are leached through the soil, there will be changes in plasticity as we pass from one subhorizon of the soil to another. In general, for soils where the maturing process is a podzolization process, the *B* horizon will be expected to be more plastic than the *A*, as the finer particles of soil accumulate in the *B* horizon. Although this is indeed the case, it is very curious that in all four of the experiments which have been completed, "sticky-point" (7, 8) measurements have shown no increase in the amount of water the soil can hold in its most plastic condition in passing from the *A* to the *B* horizon. Although there is no direct relationship between the plasticity of a soil and its water absorption capacity, one would have expected that, since the finer soil particles do, in general, hold more water than the coarser ones, the sticky-points should have followed the plasticity figures. It

[5] It seems that the ceramists are already aware that "Carbonate of Lime reduces the plasticity of clay" (6, p. 27): but the matter does not appear to have been investigated in much detail.

is not known whether this phenomenon is at all common.[6] The results of measurements on soil profile samples are given in table 2.

TABLE 2

Measurements of sticky-points and flow-plasticity figures in virgin soils

HORIZON*	$B'_{0.5}$	M†
	Penn silt loam (New Jersey)	
A_0	3.4	38.0
A_1	3.4	26.0
B_1	4.9	23.0
B_2	3.6	22.5
C	...	21.0
	Dunkirk silty-clay-loam (New York)	
A	4.6	23.5
B	6.6	23.0
C	4.6(?)	20.0
	Rough, stoney, and broken land (New York)	
A_0	4.9	....
A	6.5	....
B	7.3	....
C	3.7	....
	Hagerstown silty-clay-loam (New Jersey)‡	
A	3.0	23.0
B_1	3.8	21.0
B_2	4.8	25.0
C	4.0	25.0
	Volusia silt-loam (New York)	
A	3.2	34.0
B	3.7	31.5
C	2.7	23.0

* Since all gravel and even coarse sand has to be removed before the flow-plasticity test can be carried out, it is clear that measurements on the *C*-horizon are likely to be inaccurate. The interest lies chiefly in the *A*- and *B*-horizons.

† M = gm. of water per 100 gm. of mixture at concentration of maximum plasticity. Determined by heating in oven for 24 hours at 110°C.

‡ This soil was tested at rather too low an *A*-value. The results are not very accurate.

FLOW-PLASTICITY AS RELATED TO SOIL-TYPES

It is clear that a single measurement of the plasticity of a soil could not be related simply to a function depending on as many factors as does soil-type.

[6] Since sending this paper to press I find that this phenomenon has already been observed (see Baver, L. D., *Jour. Amer. Soc. Agron.*, 1930, 22: 935).

The plasticity would be expected to depend chiefly, if not entirely, on the texture of the soil, at any rate provided there was no great quantity of organic matter to confuse the issue. For this reason it was thought advisable to add to heavy soils, varying quantities of a standard fine sand as an inert material, thus measuring not only the flow-plasticity of the soil, but also that of each mixture, and determining the power of the soil to retain its plasticity on dilution with an inert material.[7] By plotting flow-plasticity against percentage of added sand, "dilution curves" characteristic of the soil can be obtained.

The sand is prepared in the following standard way:

Ground sand[8] contains no particle which will not pass the 100-mesh to the inch sieve, but it contains a good deal of silty material which may not be "inert" plastically. For this reason, 250 gm. of this sand was shaken in a tall cylinder, with distilled water made up to 1 liter. The mixture was allowed to stand for a quarter of an hour, and the unsettled material was then decanted, the sediment being filtered dry, then air dried, and bottled ready for use.

TABLE 3

*Soil dilution curves**

PER CENT ADDED SAND	B' FOR SOIL NUMBER				
	(1)	(2)	(3)	(4)	(5)
0	....	12.6	11.7	7.3	8.3
10	....	10.2	9.9	6.3	7.9
25	16.7	8.6	9.0	5.9	7.2
35	14.7	6.5†	7.9	5.7	6.9
50	10.5	4.9	5.8	4.6(?)	6.0
60	8.4	2.8(?)	4.9(?)	...	...
70	6.2	....	....	...	...

* Soils: 1. Whippany silty clay (N. J.). 2. Dunkirk silty clay loam (N. Y.). 3. Volusia silty clay loam (N. Y.). 4. Penn silt loam (N. J.). 5. Croton silt loam (N. J.). The soils were taken from the top few inches with no special sampling precautions, with the exception of the Penn silt loam, which was a *B*-horizon.

† This sample was 36 per cent sand.

Table 3 and figure 2 show characteristic figures and curves for a number of soils. It will be observed that these curves are remarkably linear, but, on extrapolation, do not meet at a single point. As 100 per cent sand is presumably without plasticity (or almost so), it is probable that the linearity is only apparent, and that if measurements could be made over wider ranges of sand concentration, the curves would be found to be curvilinear. It is clear that the capacity to retain plasticity on dilution does not follow hand in hand with plasticity itself. Thus two soils within the same textural group (e.g.

[7] This is not unlike the Bischof plasticity test (6, p. 47) which has been used for ceramic clays.

[8] Supplied by the Ottawa Silica Co., Ottawa, Ill.

nos. 2 and 3) may reverse the order of their plasticities on being diluted with sand, one being more plastic when both are "heavy," and the other more plastic when both are diluted. The soils chosen represent clearly marked textural types, as is plainly evident from their grouping in the figure. It is believed that these curves should be of interest in relation to soil classification.

It has been found (1) that soils which have been air dried do not always show regular "plug-flow," when made into a paste with water, and that the process of the recovery of regularity in this respect is a complex one. It is now believed by the writer that this loss of regularity is less common than was at first supposed. All of the soils used in experiments 2 and 3 were air dried, and in no case was the behavior so irregular as to prevent reliable measure-

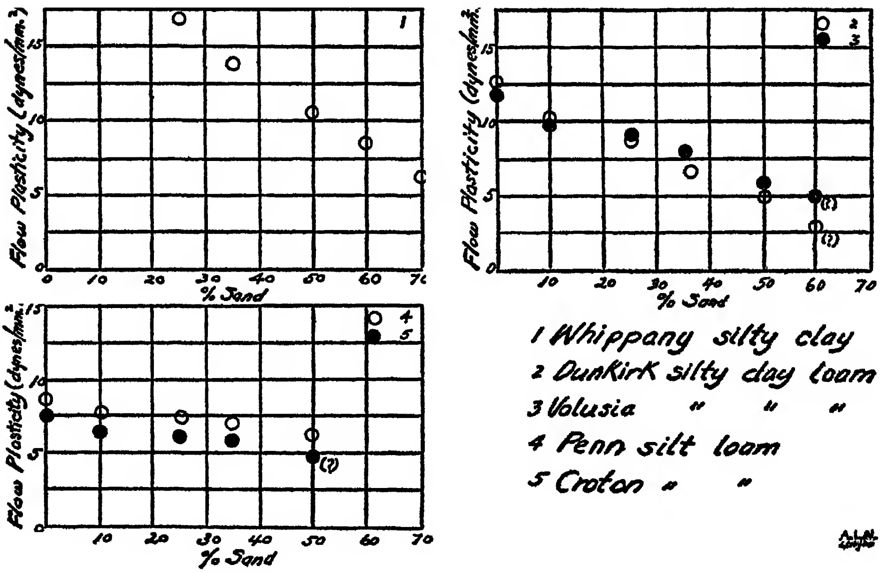


Fig. 2. Characteristic Soil Dilution Curves

ments being made, though several of the samples showed signs of irregularity. Thus the Penn silt loam pastes had to be sheared up to five or six times before a consistent, reproducible curve was obtained. At each pressure the system came to a dynamic equilibrium only after about a minute's flowing. In normal soils, this takes place within a few seconds.

SUMMARY

A brief desription is given of the way in which flow-plasticity is measured; and the relationship between flow-data in general, and plasticity in the sense defined by Wilson is discussed. It is claimed that flow-plasticity should give an interesting test for studying changes taking place in the physical properties of soils; and three cases, where changes are caused by (a) addition of chalk and

lime, (*b*) the normal leaching process, and (*c*) addition of sand to a heavy soil, have been investigated, and are quoted. It is shown that the plasticity of a soil is diminished by the addition of lime and chalk.

REFERENCES

(1) BINGHAM, E. C. 1922 Fluidity and Plasticity. McGraw-Hill Book Co.

(2) BLAIR, G. W. SCOTT 1930 The influence of the proximity of a solid wall on the consistency of viscous and plastic materials. *Jour. Phys. Chem.* 34: 1505–1508.

(3) BLAIR, G. W. SCOTT 1930 The rheology of soil pastes. *Jour. Rheol.* 1: 127–139.

(4) BLAIR, G. W. SCOTT 1930 Measurements of the plasticity of clays. *Jour. Phys. Chem.* 35: 374–382.

(5) BLAIR, G. W. SCOTT, AND CROWTHER, E. M. 1929 Flow of clay pastes through narrow tubes. *Jour. Phys. Chem.* 33: 321–330.

(6) BOURRY, E. 1911 Treatise on Ceramic Industry, ed 2 (trans. by Searle).

(7) COUTTS, J. R. H. 1929 "Single value" soil properties: A study of the significance of certain soil constants. II. Studies on Natal soils. *Jour. Agr. Sci.* 19: 325.

(8) KEEN, B. A., AND COUTTS, J. R. H. 1928 "Single value" soil properties: A study of the significance of certain soil constants. *Jour. Agr. Sci.* 18: 740–765.

(9) KEEN, B. A., AND BLAIR, G. W. SCOTT 1929 Plastometric studies of soil and clay pastes. *Jour. Agr. Sci.* 19: 684–700.

(10) SCHOFIELD, R. K., AND BLAIR, G. W. SCOTT 1930 The influence of the proximity of a solid wall on the consistency of viscous and plastic materials. *Jour. Phys. Chem.* 34: 248–262.

(11) WILSON, H. 1927 Ceramics, Clay Technology, p. 55. McGraw-Hill Book Co.

THE STIMULATION OF LEMNA MAJOR BY ORGANIC MATTER UNDER STERILE AND NON-STERILE CONDITIONS

NORMAN ASHWELL CLARK AND EMERY M. ROLLER

Iowa State College[1]

Received for publication June 12, 1930

The problem of the action of organic matter in soils and its direct influence on the green plant is by no means settled. Whether many of the essential elements are more available in organic combination, as indicated by Grandeau; whether the specific compounds released by decaying plant and animal life become available and act readily and directly on the plant as nutrients, stimulants, or poisons, as seems possible from the results of Schreiner and his coworkers at the Bureau of Soils (18, 19); whether the organic matter is the food of the microscopic life in the soil and this life forms compounds which can be assimilated by plants; or whether there are in the organic material certain "accessory substances" (4) of which minute quantities have great influence on growth, comparable with vitamins in animal life are questions to which the final answers have not yet been given.

Intimately bound up with the action of organic matter already in the soil is the function of organic fertilizers. The value of farmyard manure has been generally recognized to be greater than the value of the content of the chief elements in fertilizers. This has been ascribed to varying causes, including the increase in the bacterial activity in the soil to which manure is added. Bottomley (3) suspected the presence in the decomposing manure of accessory substances which would play an important part in the nutrition of plants. He experimented chiefly with Lemna and concluded that there existed "auximones" or growth-promoting substances, similar to vitamins and necessary for the growth of green plants.

In a previous communication (9) we reported experiments which showed that it is not necessary to add organic matter in order to obtain healthy reproduction of Lemna major (*Spirodela polyrhiza*) in solutions made from recrystallized inorganic salts and specially purified distilled water, and that the organic "auximones" postulated by Bottomley are not *essentials* for the growth of green plants. These results were later confirmed by other investigators, notably by Saeger (17), who used a diluted Knop's solution and Lemna major; by Wolfe (20), who used Shive's solutions and Lemna minor; and by Ashby in London (1) with the authors' medium and Lemna minor.

[1] Contribution from the department of chemistry. A preliminary note by the senior author on the sterilization of Lemna appeared in *Science*, 1930, 71: 268.

Although auximones were thus shown to be unlike vitamins for animals, in as much as they are not essentials for growth and reproduction, the stimulating action of very small quantities of organic substances was still unexplained. Livingston (11) pointed out the marked effect on the growth of plants of small amounts of organic matter when added to inorganic nutrient solutions; Mockeridge (14), Schreiner (19), and others have shown that decided stimulation occurred when extracts of soil or manure, or definite organic substances, were added to plant cultures. Ashby (2), who found stimulation of Lemna minor in our inorganic medium, considers the action of the organic matter to be catalytic in nature; Olsen (16) believes that it is connected with the existence of iron in organic combination in soil or manure extracts, or due to the greater availability of iron in such complex ions as those from ferric citrate. Wolfe (20) failed to get any increase in the reproduction of Lemna minor when he added a number of organic compounds to Shive's medium.

In order to investigate this stimulation of Lemna by organic substances it was necessary to obtain further information concerning the habits of the plant. The pH of the medium was determined in connection with the rate of reproduction, and an optimum of 4.8 found for the solution used (6). The plants were found to grow well under artificial light (5) without addition of organic matter. This was later confirmed by Ashby's work (1). Manganese has been claimed as an essential element for the growth of plants by McHargue and others (12). In this laboratory there was no indication that manganese was necessary for the Lemna; it was shown also that this element increased somewhat the weight of the plant and that there was an optimum concentration for this, but the manganese had no effect upon the rate of reproduction (8).

NON-STERILE EXPERIMENTS

With these details known it was possible to turn to the effect of organic matter. In the following experiments the solution contained, in millimoles per liter: 0.4 monocalcium phosphate, 8.0 potassium nitrate, 1.0 magnesium sulfate, 0.01 ferric chloride, and of manganese as manganous chloride 1 part in 10 million; the pH was kept at 4.8 by the addition of KOH where necessary. The plant cultures were grown in 250-cc. beakers in the sunlight. The beakers were placed in a thermostat which was kept at 25 C. The same technique was used for recrystallization of salts (7) and for the purification of distilled water as described previously (5). All glassware was pyrex.

The rate of reproduction is given as K in the formula

$$\log_{10} N/N_0 = K(t - t_0)$$

where N is the number of fronds at any time t (5). When log N is plotted as ordinate and t as abscissa the result is a straight line, the slope K indicating

the speed of reproduction. The "generation time"—the average time for the plant to reproduce itself—is given by the equation

$$\text{G. T.} = \frac{\log_{10} 2}{K} = \frac{0.3010}{K}$$

As the cultures were grown in sunlight, which varied from one experiment to the next, comparisons were made with controls in the inorganic medium made up as stated. The stock cultures have been growing for several years in this medium without the addition of organic matter.

Extracts were made of soil, of well-rotted farm-yard manure, and of alfalfa by maceration and twice digesting with distilled water at 70 C. for four hours with occasional shaking. The filtrates were combined and evaporated in

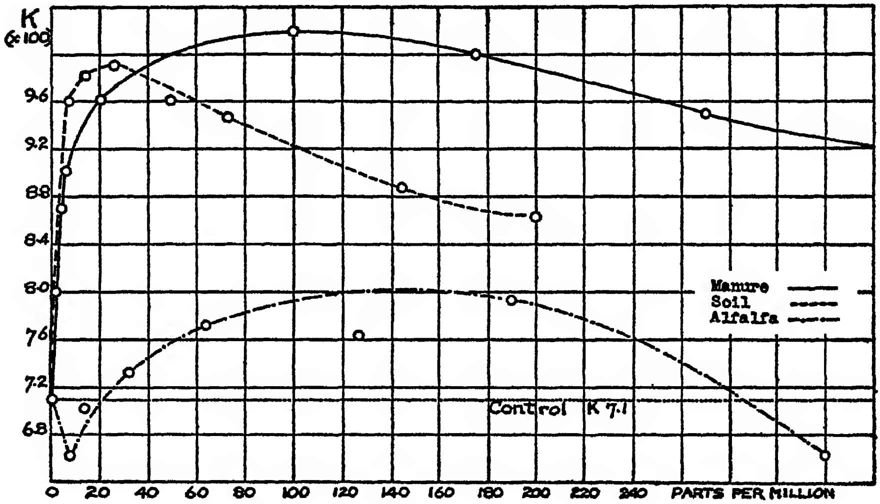


FIG. 1. RATE OF REPRODUCTION (K × 100) OF NON-STERILE LEMNA IN INORGANIC MEDIUM WITH EXTRACTS OF ORGANIC MATTER IN PARTS PER MILLION OF DRY MATTER

vacuuo at 65 C. They were then made up to a known volume and the concentration of solid matter was determined. The extracts were kept in the ice box in order to keep down the growth of microorganisms. Sufficient solution was added to the inorganic medium to give the desired amount of organic matter in 250 cc. and the pH was adjusted to 4.8. The plants were changed twice a week and the number of fronds counted.

Figure 1 shows the curves obtained when K is plotted on the number of parts per million of the three extracts. Both soil and manure extracts produced a rapid increase in the growth rate up to an optimum concentration, larger amounts lowering the speed of reproduction. Ashby (2), who used up to 20 p.p.m. of organic matter, did not find any decrease. The alfalfa curve indicates an increase in growth at some concentrations, but both large and small

amounts show a tendency to depress the rate of reproduction below the control. Signs of stimulation were observed also with extracts of yeast, carrots, and barley.

We obtained similar results in other inorganic media. Saeger (17) attempted to grow the Lemna in a solution made up of 1 gm. of each of KNO_3, KH_2PO_4, and $MgSO_4 \cdot 7H_2O$, 3 gm. of $Ca(NO_3)_2 \cdot 4H_2O$; 0.24 gm. of $FeCl_3$; in 6 liters of distilled water, but failed to get reproduction and health in the plants until the solution was diluted from 10 to 50 times. The pH of the undiluted solution, which is approximately 3.6, is much too acid for Lemna. We found that this medium had an optimum pH near 4.9 with a comparatively narrow range for good growth. When this undiluted solution was kept at the optimum pH and the manure and soil extracts were added, an increase in the rate of reproduction resulted, but it was smaller and the rise less abrupt than with our medium. The addition of asparagin and urea caused a depression of the rate at nearly all concentrations; this agreed with Wolfe's observations (20).

These results point to the conclusion that small quantities of organic extracts added to a variety of inorganic solutions can stimulate the reproduction of the Lemna not only under unfavorable conditions but also at the pH which is an optimum for growth in the inorganic solution. Bottomley (3), Mockeridge (14), Saeger (17), Ashby (2), and Olsen (16) have also reported this stimulation, and no inorganic solution has been described which will not be improved by some form and concentration of organic matter.

The addition of organic matter invariably increased the number of microorganisms in the nutrient media. In many cases the roots of the plants became coated with a fungus-like material which could not be washed off, and under the microscope showed large numbers of algae, fungi, and bacteria. To determine definitely the effect of the organic matter it seemed necessary to grow the Lemna under completely sterile conditions, so that the direct influence of different organic compounds and extracts could be studied free of bacterial action.

STERILIZATION

Hansteen (10) in 1899 attempted to sterilize Lemna minor by washing repeatedly with sterile water, and incubating in egg albumen. If there was no turbidity developed or ammonia evolved the plants were considered to be sterile. In repeating this we found that after washing the plants for one hour with sterile water, some of the albumen tubes remained clear. On transferring a small amount of the clear albumen to a nutrient agar, bacterial growth was invariably found after a few days.

Our next attempt was made by exposing the plants to ultra violet rays from a mercury vapor lamp. The technique was varied for time of exposure, distance from the light, wet and dry, in different concentrations of a large number of organic and inorganic chemicals, by alternate shaking and exposure with and without centrifuging, and by varying the temperature from the freezing point

upward. It was easy to kill the microörganisms, but with an exposure long enough to do this, the plants failed to reproduce in almost all cases. Further, there were indications that bacteria were frequently destroyed on the outside of the Lemna, but that some remained alive within the tissues. If a treated plant was dragged across the surface of an agar plate, growth often occurred only at the edges of the plant; also plates which showed no bacterial growth after several days produced colonies when the plants were macerated.

Two chemicals gave promise of complete sterilization when used with the ultra violet light. Potassium mercuric iodide K_2HgI_4 has been recommended as a germicide by Macfarlan (13) and others because the presence of organic matter does not reduce its germicidal action, and because it does not precipitate proteins. Bleaching powder was suggested as a possibility Wann.[2] Both of these chemicals, at one of the concentrations tried and with varying amounts of ultra violet light, gave a frond which remained alive and around which no bacterial growth developed on the nutrient agar. These two were therefore investigated further and it was found feasible to dispense with the ultra violet light altogether.

After treatment with the potassium mercuric iodide or the bleaching powder solution the plants were washed with sterile water and transferred to flasks containing our inorganic solution together with 1 gm. of bacto-nutrient agar per liter. In this medium at a pH of 4.8 both the non-sterile plants and bacteria developed readily. Without the ultra violet light, after 15 to 60 seconds contact with a solution of K_2HgI_4, at a concentration of 1 to 1,000 of the inorganic medium, some plants reproduced and were sterile. Under the same conditions, but for a saturated solution, the $CaOCl_2$ gave slightly better results.

The final sterilization technique was as follows: a non-sterile Lemna frond from the inorganic solution was washed with sterile water and transferred by a fork-shaped platinum wire for 30 seconds to a freshly prepared $CaOCl_2$ saturated solution. It was then washed again and placed in an Erlenmeyer flask, with the usual cotton stopper, containing the sterile medium already described. These flasks were kept at 25°C., away from direct sunlight, for five or six days, when the plants in those that appeared sterile were transferred to fresh medium. The fronds can be grown by transferring them to the pure inorganic medium directly after washing, but the bacto-nutrient agar readily shows those which are non-sterile.

From 400 plants 30 lived and were sterile. The outer part of the frond gradually withered, but the buds that were put out in the reproduction seemed to be unharmed and continued to reproduce. Transfers were made twice a week to the bacto-nutrient medium and a number of cultures inoculated into the purely sterile inorganic medium. Particular care was needed in the changing process in order to prevent contamination; the transfers were made in a small room sprayed beforehand with $HgCl_2$ solution. The plants have been

[2] Dr. F. B. Wann, in a private communication.

growing for nearly a year in the sterile inorganic medium, and they are checked periodically for contamination. No growth was found on the bacto-nutrient agar, dextrose agar, bacto-nutrient broth, and other media. Plate 1, figure 1, shows the growth on the different media after about 10 days for the non-sterile Lemna from the inorganic solution, compared to the plants from the sterile cultures. Plate 1, figure 2, shows the Lemna in the inorganic solution in the Erlenmeyer flasks.

TABLE 1

Effect of organic matter on the reproduction of sterile and non-sterile Lemna

TREATMENT NUMBER	TREATMENT OF INORGANIC MEDIUM	$K \times 100$	CONDITION AT END OF EXPERIMENT*
45	Autoclaved 20 pounds 15 minutes	5.3	s.
47	Autoclaved 20 pounds 15 minutes	5.1	s.
48	Autoclaved + 80 p.p.m. non-sterile manure extract	6.2	n.s.
49	Autoclaved + 80 p.p.m. non-sterile manure extract	6.3	n.s.
50	+ 80 p.p.m. manure extract. Autoclaved	5.3	n.s. (contaminated)
51	+ 80 p.p.m. manure extract. Autoclaved	5.4	s.
52	+ 80 p.p.m. manure extract. Autoclaved + 1 drop bacterial infusion	6.5	n.s.
53	+ 80 p.p.m. manure extract. Autoclaved + 1 drop bacterial infusion	6.6	n.s.
54	+ 80 p.p.m. manure extract. Autoclaved + 1 drop bacterial infusion	6.7	n.s.
55	Autoclaved + 1 drop bact. infusion	5.5	n.s.
56	Autoclaved + 1 drop bact. infusion	5.5	n.s.
61	+ 80 p.p.m. manure extract. Autoclaved + 1 cc. inorganic medium in which non-sterile plants had grown	6.7	n.s.
62	+ 80 p.p.m. manure extract. Autoclaved + non-sterile plants	6.9	n.s.

* s. = sterile; n.s. = non-sterile.

EXPERIMENTS WITH STERILE LEMNA

Extracts of organic matter were prepared as before but were autoclaved at 20 pounds pressure for 15 minutes. When the non-sterile plants were added to the sterilized nutrient medium containing the organic matter, an increased rate of reproduction occurred, showing that the treatment did not destroy the capacity to stimulate. Ashby (2) also has noted that autoclaving the organic extracts did not impair their stimulating properties.

Preliminary experiments with the sterile plants and organic matter showed a complete absence of stimulation over the plants in the autoclaved inorganic medium, and in several cases reproduction was slower where the organic matter

was present. When the sterile inorganic solutions containing the soil or manure extracts were inoculated with a drop of a suspension containing bacteria from the non-sterile plant, the rate of growth was increased. Indications were, therefore, that the sterile plants without organic matter grew as well or better than with the extracts in the solution, and that organic matter which increased the growth of the non-sterile plants, might depress the reproduction rate in the absence of microörganisms.

These conclusions were tested in two different experiments. In the first, a comparison was made between the inorganic medium alone, and this solution with sterile and reinoculated manure extracts. Table 1 shows the effect on

TABLE 2

Effect of organic matter and bacteria on the reproduction of Lemna

TREATMENT NUMBER	TREATMENT OF INORGANIC MEDIUM	$K \times 100$	CONDITION AT END OF EXPERIMENT*
10	Autoclaved	5.0	s.
11	Autoclaved	5.1	s.
12	Autoclaved + 1 drop bouillon infusion with non-sterile plant	4 7	n.s.
13	Autoclaved + 1 drop sterile bouillon	3.7	s.
14	+ manure extract 80 p.p.m. autoclaved	5.1	s.
15	+ manure extract 80 p.p.m. autoclaved	5.3	s.
18	+ manure extract 80 p.p.m. autoclaved + 1 drop bouillon infusion	7.3	n.s.
19	+ manure extract 80 p.p.m. autoclaved + 1 drop bouillon infusion	7.1	n.s.
16	+ alfalfa extract 130 p.p.m. autoclaved	4.4	s.
17	+ alfalfa extract 130 p.p.m. autoclaved	4.4	s.
20	+ alfalfa extract 130 p.p.m. + 1 drop bouillon infusion	6.1	n.s.
21	+ alfalfa extract 130 p.p.m. + 1 drop bouillon infusion	6.1	n.s.

* s. = sterile; n.s. = non-sterile.

the rate of growth of the Lemna. The initial pH was 4.8 and the solution was changed twice a week for five weeks. The bacterial infusion for numbers 52 to 56 was made from a 3-day inoculation of the non-sterile plants into bouillon. One drop was added to each flask when the solution was changed.

Urea, acetamide, and creatinine were also added and sterilized. The urea depressed the growth of the sterile Lemna markedly, but the acetamide and creatinine had little effect. With the addition of the bacterial infusion there was a decided depression in the reproduction rate for all three.

The figures for K show that there was an increase in the rate of reproduction when the non-sterile manure extract (nos. 48 and 49) was added to the sterilized inorganic solution (nos. 45 and 47). When this extract was autoclaved (nos.

50 and 51) there was no increase in growth unless it was reinoculated with the bacterial growth from the non-sterile plants themselves (no. 62) or from the non-sterile plants in bouillon (nos. 52, 53, and 54), or with some of the solution in which they had grown (no. 61). One drop of the bacterial infusion alone in the inorganic medium increased the rate but little (nos. 55 and 56) so that the accelerated reproduction comes from the presence of both the extract and the bacteria. The presence of the microörganisms was distinctly unfavorable with the pure organic compounds used.

The second experiment ran for four weeks. A fresh manure extract was used and the result checked the former lack of increase when the sterile extract was added to the autoclaved inorganic medium. In this group alfalfa extract was included and again inoculation was needed before stimulation occurred. Table 2 shows the treatment and the growth rate constant.

A number of pure cultures of bacteria were obtained from the bacteriological department and sub-cultured in bouillon, but no one alone was better than the plant organisms when added to the inorganic medium with 80 p.p.m. of manure extract. The following were the pure cultures used in order of increasing stimulation: *Bact. coli; Cl. sporogenes; Ps. fluorescens liquefaciens; Bact. aerogenes; Cl. Welchii; staphylococcus aureus; B. subtilis; Erythro b. prodigiosus.* The reproduction constant ($K \times 100$) varied from 5.0 to 6.7, with the sterile controls in the inorganic medium giving 5.0 and 5.1.

CONCLUSIONS

In no case have we found a stimulation of the reproduction of the Lemna in the inorganic medium, either from pure organic compounds or extracts of substances containing organic matter, when the plants are grown under sterile conditions; in some cases, especially with the organic substances like urea and creatinine, there has been a depression of the rate of growth in the sterile solutions compared with the controls in the inorganic medium. Reinoculation with microorganisms from the non-sterile plants or from the soil or manure has increased the rate of reproduction except in a few cases. Inoculation with pure cultures of bacteria has given in general an increase, although one or two did not stimulate the growth; in no case was the increase as great as inoculation with mixed cultures from the non-sterile plant.

The sterile plants have invariably reproduced slightly faster than non-sterile when no organic matter was present. Mockeridge (15) found evidences of stimulation when dead cultures of Azotobacter were added to the inorganic solution, but we have found no significant increase with either dead or living bacteria when added to the sterile inorganic medium. These facts would indicate a parasitic nature for the bacteria rather than a symbiotic relationship with the plant. If there is no organic matter present except plant tissue, this serves for the bacteria; if there is other organic matter in the medium, the bacteria attack this and in many cases produce—by decomposition or synthesis—substances which stimulate the plant. This, however, is theory and

is negatived by the fact that if sterile and non-sterile cultures in the inorganic medium are changed only once a week, the comparison soon becomes markedly in favor of the non-sterile group for general appearance. On the other hand when the sterile plants were grown on an autoclaved suspension of Carrington loam they quickly began to die, whereas reinoculation caused excellent growth and rapid reproduction.

SUMMARY

Lemna major has been grown for several years in a purely inorganic medium under non-sterile conditions.

The rate of reproduction is increased by the addition of small amounts of extracts from manure and other organic substances, but the inclusion of the organic matter increased the number of microörganisms.

Attempted methods of sterilization included ultra violet light and germicides. A technique was worked out by which K_2HgI_4 and $CaOCl_2$ sterilized the plants.

Under sterile conditions extracts of organic substances which before stimulated now had no effect or depressed the rate of reproduction. On reinoculating, stimulation occurred as before. The growth of the sterile plants was always rather faster than the non-sterile in the inorganic medium.

Pure cultures of bacteria varied in their effect in the solution containing organic matter—some increased the rate of reproduction and some did not.

None of the pure organic compounds which were tried gave any stimulation, either under sterile or non-sterile conditions.

REFERENCES

(1) Ashby, E. 1929 The interaction of factors in the growth of Lemna. 3. The interrelationship of duration and intensity of light. *Ann. Bot.* 43: 333–354.

(2) Ashby, E. 1929 The interaction of factors in the growth of Lemna. 4. The influence of minute quantities of organic matter upon growth and reproduction. *Ann. Bot.* 43: 805–816.

(3) Bottomley, W. B. 1914 Some accessory factors in plant growth and nutrition. *Proc. Roy. Soc. (London) Ser. B.* 88: 237–247.

(4) Clark, N. A. 1924 Soil organic matter and growth-promoting accessory substances. *Indus. and Engin. Chem.* 16: 249–250.

(5) Clark, N. A. 1925 The rate of reproduction of Lemna major as a function of intensity and duration of light. *Jour. Phys. Chem.* 29: 935–941.

(6) Clark, N. A. 1926 Plant growth-promoting substances, hydrogen-ion concentration, and the reproduction of Lemna. *Plant Physiol.* 1: 273–279.

(7) Clark, N. A. 1927 Purification of chemicals for use in the preparation of nutrient solutions. 1. The crystallization of phosphoric acid. *Proc. First Intnatl. Cong. Soil Sci.* 3: 300–304.

(8) Clark, N. A., and Fly, C. L. 1930 The rôle of manganese in the nutrition of Lemna. *Plant Physiol.* (In the press.)

(9) Clark, N. A. and Roller E. M. 1924 Auximones and the growth of the green plant. *Soil Sci.* 17: 193–197.

(10) Hansteen, B. 1899 Ueber Eiweissynthese in Grünen Phanerogamen. *Jahrb. Wiss. Bot.* 33: 417–486.

(11) LIVINGSTON, B. E. 1907 Further studies on the properties of unproductive soils. U. S. Dept. Agr. Bur. Soils. Bul. 36.

(12) MCHARGUE, J. S. 1927 Significance of the occurrence of manganese, copper, zinc, nickel, and cobalt in Kentucky blue grass. *Indus. and Engin. Chem.* 19: 274–276.

(13) MACFARLAN, D. 1920 The germicidal value of potassium mercuric iodide. *Amer. Jour. Med. Sci.* 159: 586–592.

(14) MOCKERIDGE, F. A. 1920 The occurrence and nature of the plant growth-promoting substances in various organic manurial composts. *Biochem. Jour.* 14: 432–450.

(15) MOCKERIDGE, F. A. 1924 The formation of plant growth-promoting substances by microorganisms. *Ann. Bot.* 38: 723–734.

(16) OLSEN, C. 1930 On the influence of humus substances on the growth of green plants in water culture. *Compt. Rend. Lab. Carlsberg* 18: 1–16.

(17) SAEGER, A. 1925 The growth of duckweeds in mineral nutrient solutions with and without organic extracts. *Jour. Gen. Physiol.* 7: 517–526.

(18) SCHREINER, O., AND LATHROP, E. C. 1909 The isolation of harmful organic substances from soils. U. S. Dept. Agr. Bur. Soils Bul. 53.

(19) SCHREINER, O., AND SKINNER, J. J. 1912 Nitrogenous soil constituents and their bearing on soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 87.

(20) WOLFE, H. S. 1926 The auximone question. *Bot. Gaz.* 81: 228–231.

PLATE 1

GROWTH OF STERILE AND NON-STERILE LEMNA

FIG. 1. Sterile and non-sterile Lemna after 10 days on different nutrient media. The culture on the extreme right is non-sterile.

FIG. 2. Sterile Lemna in inorganic nutrient solution.

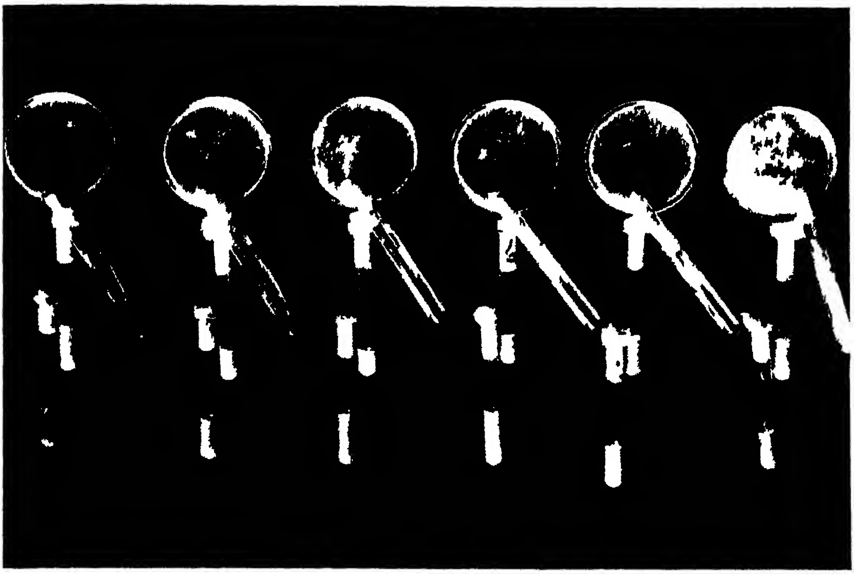


FIG 1

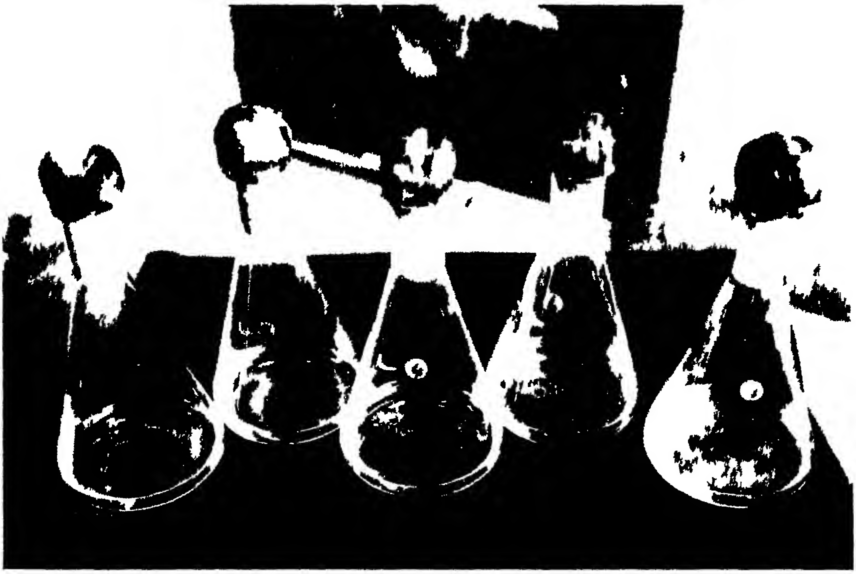


FIG 2

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: V. ION ADSORPTION AND EXCHANGE[1]

SANTE MATTSON

New Jersey Agricultural Experiment Station

Received for publication May 23, 1930

In the two preceding sections, parts III and IV of this series of papers, a number of isoelectric precipitates of aluminum and ferric "hydroxides," "silicates," "phosphates," and "humates" have been discussed in relation to the conditions governing their formation and to their composition. In the present and in subsequent sections we shall deal with the subject of ion adsorption and exchange by these materials. The isoelectric precipitates, prepared on a large scale for this work, are admirably suited, because they form natural series in which the various factors and functions vary in an ascending or descending order. Only by the use of such series will it be possible to carry out a systematic study of the phenomena of adsorption and to discover the underlying principles of such reactions.

THE CATION EXCHANGE CAPACITY OF VARIOUS COMPLEXES

We shall first study the capacity of the various precipitates for cation adsorption and exchange in neutral salt solutions. Later we shall study the adsorption of both anions and cations at various hydrogen-ion concentrations.

The isoelectrically precipitated "silicates" and "phosphates" were freed from the mother liquid by filtration but without washing. They were then dried at 65°C. and powdered. The "humates" were kept moist in the form of a stock suspension after most of the supernatant liquids were drawn off. One gram samples of the "silicates" and "phosphates" (air-dry basis) and one-half gram samples of the "humates" (105°C. oven-dry basis) were then leached on the filter each with 500 cc. neutral 0.5 *N* Ba-acetate solution then with 10 cc. *N* $BaCl_2$ and lastly washed free from Cl ions by hot water. The adsorbed Ba was then displaced with *N* NH_4Cl.

But it was found that although about 400 cc. NH_4Cl was sufficient to displace the Ba from the "silicates" and "humates," the Ba reaction persisted in the filtrate from the "phosphates" even after 1,200 cc. NH_4Cl solution had passed through. The following procedure was therefore adopted for the "phosphates:" Fresh samples were leached with 500 cc. neutral *N* NH_4-ace-

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

tate, then with 10 cc. NH_4Cl, and then washed with 90 per cent methyl alcohol [made neutral with a little ammonia as recommended by Kelly, Dore, and Brown (5)] until the disappearance of the Cl reaction. The adsorbed NH_4 ions were then displaced with $BaCl_2$ and the ammonia determined by distillation.

The results are given in tables 59, 60, and 61. The tables give the molecular ratios of acidoid (e.g. SiO_2, P_2O_5, and humus) to ampholytoid (e.g. sesquioxides) components as found in the various isoelectric systems. The

TABLE 59

Cation adsorption and exchange by isoelectrically precipitated aluminum and ferric "silicates"

SYSTEM NUMBER	COMPOSITION	ISOELECTRIC pH	LOSS ON IGNITION	SiO_2	EXCHANGEABLE Ba M.EQ./GM.	EXCHANGEABLE Ba M.EQ. PER M. MOL SiO_2
			per cent	*per cent*		
9	$Al_2O_3 \cdot (SiO_2)_{2.62}$	4.7	29.93	42.61	1.03	0.146
11	$Al_2O_3 \cdot (SiO_2)_{1.63}$	6.25	33.70	32.48	0.47	0.086
13	$Al_2O_3 \cdot (SiO_2)_{1.09}$	6.6	34.74	25.55	0.30	0.071
10	$Fe_2O_3 \cdot (SiO_2)_{2.26}$	4.95	23.60	35.22	0.98	0.168
12	$Fe_2O_3 \cdot (SiO_2)_{1.29}$	5.95	21.41	25.74	0.61	0.143
14	$Fe_2O_3 \cdot (SiO_2)_{0.79}$	6.35	20.65	18.24	0.44	0.146

TABLE 60

Cation adsorption and exchange by isoelectrically precipitated aluminum and ferric "phosphates"

SYSTEM NUMBER	COMPOSITION	ISOELECTRIC pH	LOSS ON IGNITION	P_2O_5	EXCHANGEABLE NH_4 M.EQ./GM.	EXCHANGEABLE NH_4 M.EQ. PER M. MOL PO_4
			per cent	*per cent*		
5	$Al_2O_3 \cdot (P_2O_5)_{0.857}$	4.9	29.15	38.50	1.844	0.340
7	$Al_2O_3 \cdot (P_2O_5)_{0.769}$	5.6	24.86	38.71	1.475	0.270
8	$Al_2O_3 \cdot (P_2O_5)_{0.482}$	6.45	28.40	28.68	0.875	0.216
8b	$Al_2O_3 \cdot (P_2O_5)_{0.242}$	6.65	32.00	17.10	0.300	0.125
6	$Fe_2O_3 \cdot (P_2O_5)_{0.914}$	4.0	25.20	33.50	2.370	0.503
6b	$Fe_2O_3 \cdot (P_2O_5)_{0.488}$	5.5	21.01	23.84	1.165	0.347
6c	$Fe_2O_3 \cdot (P_2O_5)_{0.244}$	6.05	19.91	14.25	0.722	0.359

"humates" however, are, expressed in terms of grams of humus to millimol sesquioxide.

Table 62 gives the exchange capacity of a series of natural soil colloids. The data are from a previous publication (9) in which the author established the relationship between the cation adsorption and the ratio of silica to sesquioxides.

The general agreement in the cation adsorbing power of the three classes of materials is highly interesting and should contribute much to an understanding of the mechanism of such reactions. The relationship of the silica/sesquioxide ratio to the cation adsorbing power, which the author has established for the

natural as well as for the synthetic "silicates" (11) applies in the same way to the "phosphates" and to the "humates." It will be noted that as the molecular ratios of P_2O_5 to R_2O_3 decrease and as the ratios of grams of humus to millimols R_2O_3 decrease the cation adsorbing and exchange capacity likewise decreases, exactly as in the silicates. This relationship appears to be quite general and might therefore be embodied in a generalized statement as follows: *The adsorption and exchange of cations, at pH 7.0, by soil colloids, in the same*

TABLE 61

Cation adsorption and exchange by isoelectrically precipitated aluminum and ferric "humates"

SYSTEM NUMBER	COMPOSITION: $\frac{\text{GRAM HUMUS}}{\text{M. MOL } R_2O_3}$	ISOELECTRIC pH	PER CENT HUMUS (105° DRY BASIS)	EXCHANGEABLE Ba M.EQ./GM. b	EXCHANGEABLE Ba ON BASIS OF FREE HUMUS M.EQ./GM. a	EXCHANGE-MILLI-EQUIV. HUMUS IN COMBINATION WITH Al OR Fe $a - b$	EXCHANGE-EQUIVALENTS HUMUS IN COMBINATION WITH 1 MOL R_2O_3	PER CENT FREE HUMUS $\frac{b \times 100}{a}$
52	$Al_2O_3 \cdot (Hum)_{1.305}$	4.5	91.6	1.827	2.62	0.79	1.13	70.0
53	$Al_2O_3 \cdot (Hum)_{0.533}$	5.15	81.7	1.055	2.33	1.27	0.83	45.4
54	$Al_2O_3 \cdot (Hum)_{0.265}$	5.5	68.9	0.600	1.97	1.37	0.53	30.5
56	$Al_2O_3 \cdot (Hum)_{0.140}$	6.2	53.9	0.411	1.54	1.13	0.29	26.7
58	$Al_2O_3 \cdot (Hum)_{0.070}$	6.8	36.8	0.332	1.05	0.72	0.14	31.6
55	$Fe_2O_3 \cdot (Hum)_{0.277}$	4.55	60.5	1.330	1.73	0.40	0.18	77.0
57	$Fe_2O_3 \cdot (Hum)_{0.141}$	5.4	43.8	0.848	1.25	0.40	0.13	67.8
59	$Fe_2O_3 \cdot (Hum)_{0.070}$	5.95	27.9	0.553	0.80	0.25	0.06	69.2
	Free humus	....	....	2.857	....	....	....	....

TABLE 62

Cation adsorption and exchange by natural soil colloids

COLLOID	COMPOSITION SiO_2/R_2O_3	SiO_2	EXCHANGEABLE Ba M.EQ./GM. (108° DRY BASIS)	EXCHANGEABLE Ba M.EQ. PER M. MOL SiO_2
		per cent		
Bentonite	3.81	48.80	1.102	0.136
Fallon	3.82	52.57	0.947	0.109
Sharkey	3.18	52.05	0.796	0.092
Marshall	2.82	45.93	0.671	0.088
Sassafras	1.89	41.14	0.331	0.049
Norfolk	1.63	39.25	0.207	0.032
Aragon	0.55	15.86	0.164	0.062

state of aggregation increase with the increasing ratio of the acidoid to the ampholytoid constituents of the colloid.

It would seem that this general relationship can best be accounted for on the assumption that the acidoid and ampholytoid constituents exist in partial combination with one another in the form of compounds which are at the same time both acidic and basic, and that the uncombined acidoid valences constitute the seat of cation adsorption and exchange whereas the free basoid mol fraction is responsible for the adsorption of anions. We shall proceed on this assumption.

Since basoid colloids, that is, materials which would react with only anions at any pH, are unknown, the term "ampholytoid" is applied to the basic constituents. The sesquioxides are, as we have seen, ampholytoids and become strong acidoids in alkaline solutions. Lateritic soil colloids which show a very low cations exchange capacity at pH 7.0 adsorb, for this reason, great quantities of bases from alkaline solutions (10). The preceding statement applies therefore to pH values below that of the isoelectric point of the sesquioxides.[2]

The cation adsorption per gram colloid is, however, not a linear function either of the acidoid/sesquioxide ratio, which we would also not expect, or of the percentage of acidoid in the complex. There are two conditions which would cause the adsorption to depart from a linear relationship to the percentage of acidoid. In the first place the state of aggregation in the different materials might not be such as to leave the same proportions of the acidoid valences free and active. In the second place, and this is more certain, the acidoid and the ampholytoid constituents do not themselves enter into combination with one another to the same relative extent in the various complexes. Thus the number of free acidoid valences which are not in combination with the (by ordinary salt reactions) non-exchangeable ampholytoid, are not proportional to the total acidoid present, but vary with the acidoid/ampholytoid ratios.

This becomes quite evident when we calculate the exchangeable milliequivalent cations per millimol SiO_3 and PO_4 as in the last columns in tables 59, 60, and 62, and the percentage free humus as in the last column in table 61. It will be noted that in the colloids having a high ratio and formed at a low pH, the exchange per millimol SiO_3 and PO_4 is the greatest. This means obviously that a larger molar fraction of the acidoid remains free to enter into exchange reactions in the case of the more highly silicated and phosphated complexes.

The same holds for the humus complex, in which the percentage of free humus increases with an increase in the composition ratio as the isoelectric pH is decreased. The quantity of humus that has entered into combination with each mol sesquioxide is also greater the lower the isoelectric pH, just as in the case of the mineral complexes. We do not know the molecular weight of the humus complex, which obviously is an aggregation of several molecules, nor do we know its true equivalent weight as an acidic entity but we know its exchange-equivalent in the free state, which is 2.857 milliequivalents per gram. Knowing the percentages of humus in the various complexes, we can calculate, from their exchange capacities, the exchange—equivalents of humus which have combined with, or been inactivated by, the aluminum and iron. The results are shown in table 61.

[2] The methods of Hissink for determining the total saturation capacity T and the derived value $\frac{S:100}{T}$ assume therefore absurd proportions when applied to lateritic soils.

The number of exchange-equivalents of humus in combination with each mol of sesquioxide, which vary between 1.13 and 0.06, are apparently smaller than the number of equivalents of SiO_3 and PO_4 so combined. But it is possible that that part of the hydrogen which dissociates too slightly to be displaced by the cations of the neutral salt solution has been displaced by aluminum and iron. It is therefore impossible, by this method, to tell anything about the exact extent to which the humus occupies the valences of these ions. This applies to the silicated and phosphated complexes as well. We can not merely subtract the exchange-equivalents from the total equivalents of SiO_3 and PO_4 and say that the remainder is in combination with Al and Fe. Both of these anions exist in combination with hydrogen so slightly dissociated as not to be displaced by the cations of the neutral salt solution. Then there is the possibility, which, in the case of the natural colloids, is a certainty, that some of the otherwise (in exchange reactions) active acidoid valences are locked up in the interior of the particles. Kelly, Dore, and Brown have shown that if the natural colloids be sufficiently ground, all of the mono- and divalent metal cations are exchangeable (5).

The "non-exchangeable" cations present in natural colloids and which make up a general average of about two-thirds to three-fourths of the total mono- and divalent bases present (1) are therefore non-exchangeable because of their position within the crystal lattice or molecular aggregates. The synthetic "silicates" which have not had time to crystallize show for this reason a higher exchange-capacity. Their exchange capacity is even higher in the moist condition than when dried at 65°C., which was done with the intention of increasing their stability.

Comparing the exchange capacities of the synthetic colloids, we note that the phosphated and humated complexes possess the highest capacities, especially where the composition ratios are high.

A very interesting and instructive observation is made by comparing the exchange capacities of the aluminum and ferric complexes. The ferric complexes show a much greater power to adsorb and exchange cations. Although the percentage of acidoid is lower in the ferric than in aluminum complexes of the same molecular ratios, because of the high molecular weight of Fe_2O_3, the exchange capacities per gram are uniformly higher in all ferric complexes. The differences are best seen by comparing the exchangeable cations per millimol SiO_3 and PO_4 and the percentages of free humus. Only the last three aluminum "humates" are to be compared with the three ferric "humates," which have the same composition.

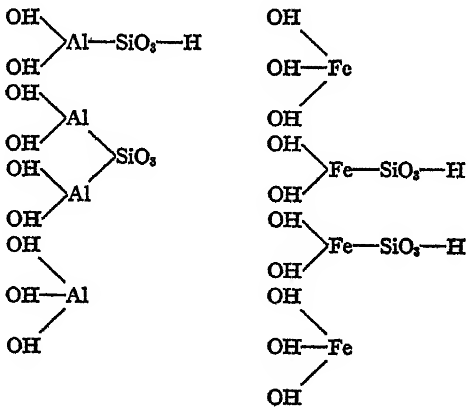
THE MECHANISM OF ION EXCHANGE

The cause of this difference in exchange capacities between the aluminum and ferric complexes is reflected in the exchange equivalents of humus in combination with one mol sesquioxide. For the last three aluminum "humates" the figures are 0.53, 0.29, and 0.14 as compared to 0.18, 0.13, and 0.06 for the ferric "humates." This means that the ferric compounds are

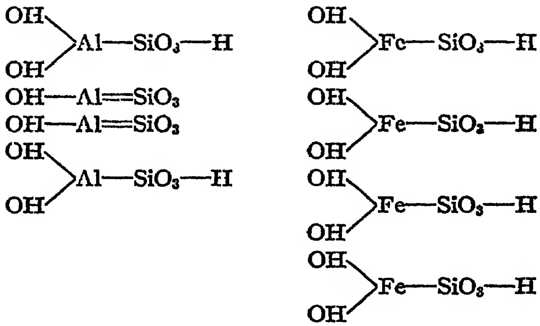
more hydrolyzed, that is, the OH ions displace more energetically the valences of other anions in the ferric than in the aluminum compounds. Now since these other anions are di- or polyvalent, one or more of their valences will be more easily displaced than the remainder. They are, therefore, not always bodily displaced from the complex but suffer only a partial displacement. *The free valences of these anions, partially linked up in stable combination constitute the seat of cation adsorption and exchange.*

The amphoteric colloids here studied represent salts of weak acids aud bases containing residual H and OH ions. Their H and OH ion product must be less than the ion product of water ($K_w = 1 \times 10^{-14}$). *The strength of the acid and basic residue depends upon the dissociation constant of each. Where the one is high the other must be low.* $Fe(OH)_3$ *is a weaker base than* $Al(OH)_3$. *The former leaves therefore a stronger acid residue in the various amphoteric complexes. The ferric "phosphates," "silicates," and "humates" have therefore a higher cation exchange capacity because they contain more displaceable H ions and they have a lower isoelectric pH because they contain more active H ions than the corresponding aluminum complexes.*

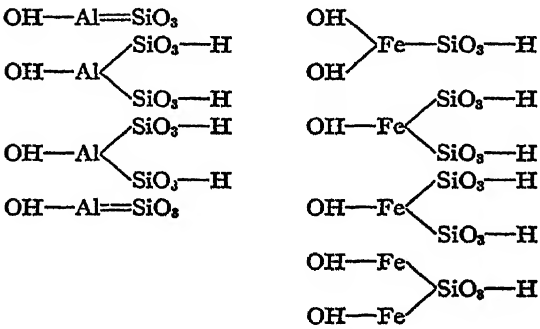
All this can hardly be represented by any formula, but it will be useful to indicate the structure of the various complexes in order to account for the differences in the exchange capacities according to the conception here developed. The amphoteric complexes will be represented in the acidoid-basoid form, that is, in the hydrogen-hydroxyl saturated condition. Either of these ions may, of course, be partially displaced by other cations and anions respectively. This happens to some extent even at the isoelectric point, as has been shown. At pH values above this point the progressive displacement of the H ion by metal cations increases rapidly. At pH values below the isoelectric point the OH ions are displaced by other anions. The following formulas, of course, represent nothing exact, but are merely meant to account for the general differences in the adsorbing power:



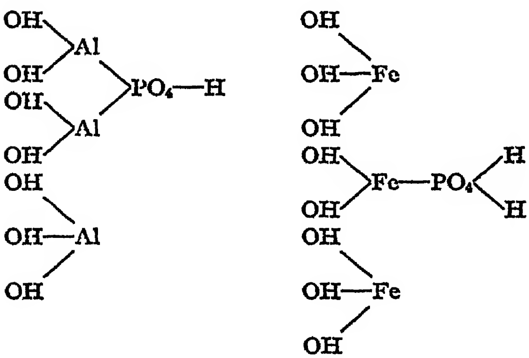
$\frac{SiO_2}{R_2O_3} = 1$ Cation adsorption low
Anion adsorption high



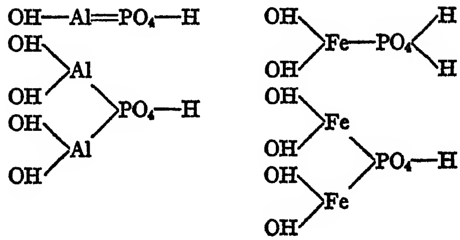
$\frac{SiO_2}{R_2O_3} = 2$ Cation and anion adsorption intermediate



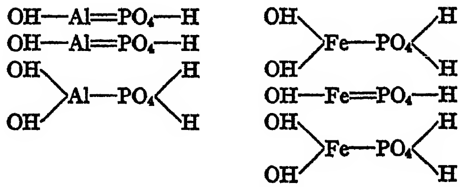
$\frac{SiO_2}{R_2O_3} = 3$ Cation adsorption high
Anion adsorption low



$\frac{P_2O_5}{R_2O_3} = \frac{1}{3}$ Cation adsorption low
Anion adsorption high



$\frac{P_2O_5}{R_2O_3} = \frac{2}{3}$ Cation and anion adsorption intermediate.



$\frac{P_2O_5}{R_2O_3} = 1$ Cation adsorption high
Anion adsorption low

These formulas represent molecular ratios which can be expressed in whole numbers. Any intermediate composition is possible if a sufficient number of molecules be considered.

The author has shown (10) that while the adsorption of the cations increases with the silica sesquioxide ratio in the natural colloids, the anion adsorption increases in the reverse order. The preceding formulas account for this behavior because the acidity and the basicity of the complexes, as here presented, vary in the inverse order. It will be seen that, in the assumed structure, the ferric complexes are at the same time more acidic and more basic than the corresponding aluminum complexes. This is all in accordance with their behavior.

Some soil investigators (6) would ascribe soil acidity and cation exchange to the existence of an alumino-silicic acid of a definite composition. The author cannot, on experimental as well as on theoretical grounds, share in this viewpoint. We have here before us three different series of colloidal aluminum and ferric compounds, all very different in the nature of their acid radicles and which show the same general behavior, chemically as well as electrokinetically, as the natural soil colloids. Yet it is doubtful whether anyone would ascribe these properties to the formation of any particular alumino-or ferric-silicic, phosphoric, or humic acids. *It seems quite obvious that we are dealing with colloidal complexes represented by a continuous gradation in composition as well as in properties.*

It is true that soil colloids differ from the synthetic products by being more

resistant to the action of acids and alkalies. But this does not prove, as has been maintained,[3] that the former are different compounds from the latter. Single substances often show very great differences in resistance to the action of solvents. Ageing, which brings about a change in the state of aggregation, may alone be responsible. Soil colloids are at least to some extent crystalline, as has lately been brought out by two interesting investigations by Hendricks and Fry (4) and by Kelly, Dore, and Brown (5). The crystalline materials appear to differ, as does the composition of the colloids, with the climatic conditions and the degree of weathering. The composition of the crystal is evidently affected by the composition of the complex from which it is formed. The analysis of "purified" materials has failed to show a stoichiometrically definite composition. This is not surprising, for although a constant ratio probably obtains within any one crystal, such will not be the case in the adsorption layer, which is very large in highly dispersed materials. On the surface of a silicate crystal a large number of the silicate ions will be displaced by phosphate, humate, hydroxyl, and a host of other anions.

A complete displacement (as distinguished from a fractional displacement) of silicate ions by OH ions and the subsequent loss of the former from the soil must lead to a decrease in the cation adsorption and exchange power, because the adsorption layer will then largely consist of free sesquioxides which do not combine with cations at a pH of 7.0. This condition is exemplified by the highly weathered tropical or subtropical soil colloids. These have a low silica/sesquioxide ratio and a low exchange capacity and are yellow or red as a result of free ferric oxide.

The sesquioxide layer on the surface of the particles formed by this displacement may be removed by peptisation with a hot, saturated $AlCl_3$ solution. An ash-gray silicate residue is then obtained in which the silica/sesquioxide ratio is higher and which possesses a higher cation adsorption and exchange capacity. Six soil colloids, all with a silica/sesquioxide ratio below 2.0 and of a rusty red color, except the Norfolk which is yellow, were thus treated. Following the treatment the $AlCl_3$ was removed by washing with 0.05 N HCl. The results are shown in table 63.

Whether the treatment lasted 3 days or 60 days made little difference. In all cases the composition of the ash-gray residues is not very far removed from a silica/sesquioxide ratio of 2.0, which is that of kaolinite and halloysite. X-ray analysis has also indicated, in highly weathered colloids, a structure similar to that of halloysite (4, 5).

The cation exchange capacity of the ash-gray residue was, in the case of the Norfolk colloid, 0.425 as compared to 0.207 milliequivalents per gram in the original material, an increase of 100 per cent. The power to adsorb anions such as the Cl and SO_4 ions from acid solution was, on the contrary, com-

[3] By Dr. E. Truog at the 1928 meeting of the American Society of Agronomy. Washington, D. C.

pletely destroyed by the treatment (10). From this it is quite obvious that the cation adsorption is caused by a combination with the acidic constituent while the anion adsorption is brought about by a combination with the basic or ampholytoid constituent.

A displacement of the silicate ion by the phosphate might be expected to increase the cation exchange capacity since the phosphated complex possesses this capacity to a higher degree. A displacement of a di- or polyvalent anion by a monovalent anion should theoretically reduce the cation exchange capacity, since a monovalent anion in combination with the ampholytoid would have no free primary valence available for the exchange. Residual valences may, however, be active. Experiments are in progress to test this.

A fractional displacement of the silicate ion without its complete detachment from the complex and removal from the colloid should increase the cation exchange capacity because this would result in a greater number of free acidoid valences. The "build-up" in the exchange capacity of orthoclase and of artificial and natural colloids in alkaline solutions as reported by

TABLE 63

The silica/sesquioxide ratio of soil colloids before and after treatment with a hot, saturated $AlCl_3$ solution

TREATMENT	SILICA/SESQUIOXIDE RATIOS OF SOIL COLLOIDS					
	Aragon	Cecil	Norfolk	Orange-burg	Chester	Sassafras
None	0.55	1.34	1.63	1.71	1.79	1.89
$AlCl_3$, 3 days	2.08	1.81	2.19	1.98	2.08	2.13
$AlCl_3$, 60 days	2.17	1.92	....	2.08	2.18	2.33

Breazeale and Magistad (2), Burgess (3), and Magistad (8) may be thus explained. The OH ions partially displace the silicate ions from their union with aluminum and iron, giving rise to a greater number of free acidoid valences. Treating the Sassafras colloid for one hour on the steam bath with *N* NaOH and then titrating back to neutrality with HCl, increased the exchange capacity of the colloid about 300 per cent. The sample so treated was much more soluble in 0.05 *N* HCl than was the untreated colloid, pointing to an unlocking of the silicate bonds according to the following scheme:

$$OH{-}Al{=}SiO_3 + NaOH$$

$$\begin{matrix}OH\\OH\end{matrix}\!\!>Al{-}SiO_3{-}Na$$

If the cation exchange capacity depends upon the number of free silicate or other acidoid valences, then the entirely free silica gel should possess a higher cation exchange capacity than that of any silicate, just as the free

humus possesses a higher exchange capacity than any of the humates. But silica gel, as ordinarily prepared, possesses a very moderate cation exchange capacity. For dried silica gel an exchange capacity of only 0.236 milliequivalent per gram was found.

It was believed that a structure fine enough to prevent the free movement of the common, hydrated cations into the interior of the gel may be responsible for this phenomenon To study this possibility 1 gm. of silica was precipitated from Na_2SiO_3 by neutralization with HCl in the presence of 10 gms. of cane sugar in a small volume of water. After being dried on the steam bath the gel was washed and treated in the usual manner with Ba-acetate. It was thought that if the gel were dehydrated in the presence of the large sugar molecules a coarser structure should result. This was also indicated by the great volume of this gel as compared to the sample dried in the absence of sugar. The exchange capacity was also increased by the treatment but only to 0.610 milliequivalent per gram, or about 150 per cent. A very large part of the silica was therefore inactive. How much of this inactive part exists in the anhydride form and how large a fraction of its displaceable hydrogen remains undisplaced at a pH of 7.0 we do not know. But it is quite obvious that the closeness in structure of nondispersing materials like silica gel and the permutites affects the penetration of ions. Wiegner and Muller (14) found only a very slight adsorption of methylene blue by the permutites whereas in the case of the high-disperse soil colloids the adsorption of this dye is considerable and proportional to their cation exchange capacity, as has been shown by the author (9). A lump of silica gel becomes dyed only on its surface when placed in a solution of methylene blue.

We have seen that the removal of sesquioxides by petisation with an $AlCl_3$ solution results in an increase in the cation exchange capacity and also that this capacity is increased by treatment with alkalies, which increase is believed to be due to a liberation of an additional number of silicate valences through a displacement from their combination with aluminum and iron by the OH ions. We shall now see that the opposite process—the introduction of additional quantities of aluminum and iron into the exchange complex—results in a lowering of the cation exchange capacity. This would, of course, follow from the exchange capacities of the various complexes studied but we shall use a natural colloid in order to show that what is true for the one holds for the other group of materials.

In the previous section 1-gm. bentonite samples were isoelectrically flocculated with 4.0 millimols $AlCl_3$ and $FeCl_3$ respectively. This means that 0.2044 gm. Al_2O_3 and 0.320 gm. Fe_2O_3 entered each gram of bentonite. The isoelectric pH values were 6.75 and 6.5 respectively.

The cation exchange capacities in milliequivalents per gram bentonite were as follows:

Original betonite	*Bentonite — Al_2O_3 complex*	*Bentonite — Fe_2O complex*
1.002	0 429	0 970

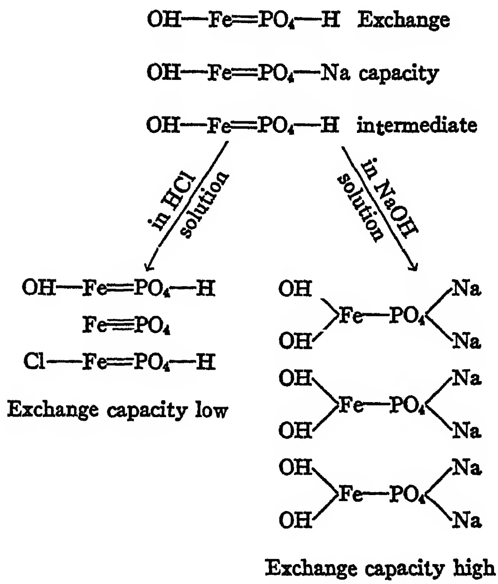
The aluminum ion has combined with and made inactive 57 per cent of the active silicate valences in the bentonite whereas the ferric ion has only inactivated about 3 per cent. The cation exchange capacity is therefore higher in the ferric complex than in the aluminum complex, just as we found it to be in the silicated, phosphated, and humated compounds of aluminum and iron. In the aforementioned complexes the bentonite plays the part of the acid anions of the previous experiments. It is also in reality a complex silicate ion. The exchangeable cations have been partly displaced by the Al and Fe ions which are not re-displaceable by the neutral salt treatment. The aluminum ions are more active in this respect than the ferric ions because the OH ions displace the silicate ions from their ferric combination more energetically, thus leaving a greater number of free silicate valences in the ferric complex.

By an alkaline solution it would be possible to displace a large part of the trivalent cations from their silicate combination and thus restore the exchange capacity of the bentonite. Upon acidifying, a recombination will, however, take place, the extent of which will depend upon the pH. A shifting in bonds must take place with each change in the H-ion concentration. The reaction between colloidal ionogens is, however, slow, because of their non-diffusibility. Equilibrium proceeds slowly, therefore, in the wake of any change in the ionic environment. Thus the cation exchange capacity will be different depending upon whether we start the neutral salt treatment on acid or alkaline materials. On the acid side the number of bonds between acidoid and ampholytoid is greater than on the alkaline side. At the neutral point the number of such bonds is intermediate, but since equilibrium is slowly attained we get a higher exchange capacity at a pH of 7.0 when we approach this point from the alkaline than when we approach it from the acid side.

The influence of a change in the pH value upon the shifting of bonds may be illustrated in the case of a ferric "phosphate" complex having the composition shown at top of page 323.

A similar displacement takes place in the "silicates" and "humates." In the ferric "phosphate" complex this change can be observed by the change in color.

This conception of the colloidal complex explains a number of phenomena. It explains the great adsorbing power for bases from alkaline solutions and it explains the fact observed by the author (11) that the OH ion enters the complex together with the cation. From what has been said it is clear that the exchange capacity of an amphoteric colloidal complex does not represent a constant quantity even at the same pH. The exchange capacity depends upon the manner in which the valences are linked within the complex. Any treatment, physical or chemical, may bring about a shifting in the bonds The soil colloidal complex is a dynamic thing and is never the same under any two sets of conditions.



THE RELATION BETWEEN THE ISOELECTRIC POINT AND ION EXCHANGE

In the foregoing we have studied the cation exchange at a pH of 7.0. We have seen that the lower the isoelectric pH of the colloid, that is, the farther removed this pH is from pH 7.0, the greater the cation exchange capacity. Aluminum "hydroxide" (precipitated from the chloride) is isoelectric at or about pH 8.0. It is electropositive at pH 7.0 and showed no trace of cation exchange at this pH. How is the ion exchange related to the isoelectric point? What happens to the ion exchange, anion as well as cation, as we approach and pass this point from the negative to the positive side or vice versa? Since quantitative experiments dealing with these questions will be presented and discussed in the succeeding section we shall confine ourselves in the remaining space to a few qualitative experiments.

A number of aliminum "silicate" suspensions were prepared by mixing 100 cc. containing 1.0 millimol $AlCl_3$ with 100 cc. containing 1.5 millimol Na_2SiO_3. The pH was adjusted to cover a wide range by adding NaOH to the silicate or HCl to the chloride before the mixing of these solutions. The degree of flocculation was noted and cataphoresis measurements were made as shown in table 64. To 20-cc. portions of the various suspensions were then added 5 cc. water in one of the series and 5 cc. normal salt solutions in other series, and the pH determined colorimetrically in each as shown.

We note that all the salts give rise to an exchange acidity in the strongly negative suspensions whereas in the strongly-positive suspensions an exchange alkalinity is developed. The power of the various cations to displace hydrogen is, as far as the pH values are accurate, the following:

$$Li < Na < K = Mg < Ca < Ba$$

The power of the anions to displace the OH ions is, for the two ions used:

$$Cl < SO_4$$

How far the two kinds of displacements overlap can be learned only by a quantitative determination of all the ions in the solution at equilibrium. This work will be reported later. It will be noted that the strongly adsorbed SO_4 ions develop an exchange alkalinity at a pH at which the original suspension is still highly negative. It is obvious that the charge of the colloid was affected and even that the isoelectric point was displaced by the various salts. In the presence of so much electrolyte the cataphoresis cannot be determined

TABLE 64

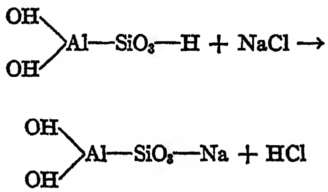
The exchange reaction at various pH values in a negatively and positively charged aluminum "silicate" and the lytropic effect of the ions

NUMBER	0.1 N NaOH	0.1 N HCl	FLOCCULATION	μ/SEC. 1 VOLT/CM.	pH after 5 cc. water or N salt solution was added to 20 cc. of the various suspensions:							
					Water	LiCl	NaCl	KCl	$MgCl_2$	$CaCl_2$	$BaCl_2$	Na_2SO_4
	cc.	*cc.*										
1	2.0	....	0	......	7.8	7.4	7.2	7.0	7.0	6.95	6.8	7.2
2	1.0	....	0	......	7.4	...	6.9	...	...	....	6.55	6.95
3	...	1.0	0	......	6.7	...	6.55	...	...	....	6.3	6.55
4	...	2.0	Slow	−2.52	6.45	...	6.3	...	...	....	6.15	6.35
5	...	3.0	Instant	−2.24	6.2	...	6.1	...	...	....	5.95	6.17
6	...	3.4	"	−1.21	5.7	...	5.6	...	...	....	5.45	6.0
7	...	3.6	"	+0.74	4.95	...	5.0	...	...	....	4.95	5.4
8	...	3.8	"	+1.38	4.75	...	4.95	...	...	....	4.85	5.3
9	...	4.0	"	+1.59	4.5	...	4.85	...	...	....	4.85	5.1
10	...	5.0	Slow	+1.92	4.3	...	4.6	...	...	....	4.62	4.95
11	...	6.0	0	+2.27	4.2	...	4.5	...	...	....	4.6	4.8
12	...	10.0	0	......	3.9	...	4.3	...	...	....	4.38	4.6

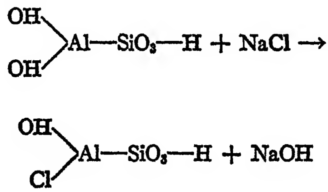
without non-polarizing electrodes. But it is certain, from what has already been shown in the case of sulfated systems, that the SO_4 ion did not displace the isoelectric point to a higher pH but rather to a lower value. The cataphoretic isoelectric point is therefore no dividing line between exchange acidity and alkalinity. The point at which the exchange acidity changes to an exchange alkalinity, or vice versa, which we might call the point of exchange neutrality, will depend upon the relative displacing power of the two ions of the salt. The cataphoretic isoelectric point we have assumed to represent that point at which the anionic and cationic dissociations are equal. (The isoelectric point is possibly also determined by the orientation of the solvent molecules at the interface.) The position of either point is therefore determined by the dissociation constants of the amphoteric complex formed with

the two ions. But the effect is such that an ion which displaces the point of exchange neutrality in one direction must displace the point of electric neutrality in the opposite direction. Thus a slightly dissociating, and therefore strongly displacing, anion like the SO_4 ion, will carry the point of exchange neutrality to a higher pH but will displace the isoelectric point to a lower pH because of a suppressed anionic dissociation. A slightly dissociating, and therefore strongly displacing, cation will have the opposite effect.

The exchange acidity formed at high pH values may be represented thus:



and the exchange alkalinity which is formed at low pH values thus:



We have now seen that an amphoteric colloid like aluminum "silicate" manifests an exchange acidity at higher pH values whereas at lower pH values the neutral salt reaction passes through a point of exchange neutrality and then develops into an exchange alkalinity, that is, cation exchange predominates at high pH values whereas anion exchange predominates at low pH values. An important question now is this: does this behavior, like so many of the others that we have studied, apply to the natural soil colloids as well as to the synthetic materials? Theoretically the amphoteric soil colloids, which possess a low silica/sesquioxide ratio and have an isoelectric point within the usual range of soil reaction, should show an exchange alkalinity at low pH values. There are a number of such colloids especially in the lateritic type of soils. One such soil, the Nipe clay from Oriente Cuba[4] has been studied in respect to this behavior. The electrodialyzed soil had a pH of 5.95 in water and a pH of 6.5 in N Na_2SO_4. When the same sample was treated with 0.1 milliequivalent HCl per gram soil the pH values were 3.6 in water and 5.1 in 0.5 N Na_2SO_4. The exchange alkalinity was therefore considerable especially in the more acid condition.

[4] Analyzed by Glenn Edgington and kindly supplied the author by W. O. Robinson, both of the Bureau of Chemistry and Soils. This soil contained 10.19 per cent SiO_2, 15.84 per cent Al_2O_3, and 62.51 per cent Fe_2O_3.

The significance of this behavior in connection with certain methods of determining soil acidity, "avidity" of the soil acids and lime requirements, will readily be recognized. An acid soil having a high sesquioxide content will either show an apparent exchange acidity which is smaller than the real or it will show a negative exchange acidity when in equilibrium with a salt solution, as a result of a displacement of OH ions by the anions of the salt. Upon prolonged leaching with a neutral or alkaline solution the adsorbed anions will, however, be largely or completely redisplaced by the firmly associating OH ions.

Soils in which the colloidal complex has a low silica/sesquioxide ratio and low cation exchange capacity have a higher pH when completely unsaturated with bases than do soils in which the aforementioned ratio and capacity are high. At a given pH, say about 5.0, the degree of base saturation is much lower in the former than in the latter type of soils. There is, therefore, no correlation between the pH and the degree of base saturation, as has recently been conclusively shown by Pierre and Scarseth (13). All this and many other phenomena discussed in the literature become comprehensible in the light of the behavior of the variously composed complexes. A complex having a low silica/sesquioxide ratio, and a low cation exchange capacity at a pH of 7.0 is not, at this point, far removed from its isoelectric point. At or slightly below the isoelectric point the cation exchange capacity must approach the vanishing point. This explains the low exchange capacity of such colloids at a pH of 7.0, it explains the rapid decrease in the degree of base saturation with a decrease in pH, and it explains the high pH at complete unsaturation, all as compared to colloids having a high silica-sesquioxide ratio and which have no isoelectric point, i.e., are not amphoteric, or have this point at a very much lower pH.

It will be recalled that the higher the acidoid/ampholytoid ratio the greater is the free acidoid mol fraction as measured by the number of milliequivalents exchangeable cations per millimol SiO_3 and PO_4. This evidently means more active acidoid hydrogen in the high ratio complexes because the least active hydrogen will be the last to be displaced by the ampholytoid valences. The unsaturated, highest ratio complexes react therefore most acid. In the foregoing the silicate ion has been represented as divalent, but it may be tetravalent like $H_4Si_3O_8$ or even higher associations. We may then account for all degrees of active hydrogen depending upon the (average) number of ions displaced by aluminum and iron.

THE DONNAN EQUILIBRIUM IN AN AMPHOTERIC COLLOID

In the electronegative condition the colloid particles are surrounded by a swarm or atmosphere of cations which have dissociated from the acidoid group of the colloid. These ions are hydrogen and metal cations, the relative proportion of each depending upon the degree of base saturation, i.e., upon the pH. In the electropositive condition the ion atmosphere consists of anions dis-

sociated from the basoid group of the colloid. If the colloid has been acidified with HCl then the Cl anions will predominate.

Now this difference in the composition of the micellar atmosphere will express itself in the unequal distribution of an ion, the H ion for example, within a suspension as compared to a clear filtrate or to an outside solution in equilibrium with the suspension. This distribution will be according to the Donnan equilibrium and will be such that the negative suspension, having H ions in the micellar atmosphere, will have a lower pH than the outside solution whereas the positive suspension, having Cl ions in the micellar atmosphere, will have a higher pH than the outside solution in equilibrium or, what is the same thing, the filtrate from the suspension.

The reasons for this difference have been explained in part I (12) of this series but will be here briefly recapitulated: In the micellar solution of the electronegative colloid there is a certain concentration, z, of H ions belonging to the micelle and which, for electrostatic reasons, are unable to diffuse into the external solution. Then there is a certain concentration, y, of free H ions belonging to the free acid, HCl in this case, and of course, an equal concentration, y, of free Cl ions. In the intermicellar solution we have only free H and Cl ions, the concentration of each being x.

Now the Donnan equation demands that the product of the concentration of any pair of diffusible ions must be the same in any part of the system at equilibrium. Hence

$$(x_{H}\text{ outside})\ (x_{Cl}\text{ outside}) =$$

$$(y_{Cl}\text{ inside})\ (y_{H} + z_{H}\text{ inside})$$

or

$$x^2 = y\,(y + z)$$

Since

$$(y + z) > x$$

$$C_{H}\text{ inside} > C_{H}\text{ outside}$$

or

$$\text{pH inside} < \text{pH outside}$$

In the positive suspension where the micellar atmosphere consists of anions, e.g., Cl ions, there will be a concentration, z, of Cl ions belonging to the micelle, a concentration, y, of free Cl ions belonging to the free acid and an equal concentration, y, of free H ions. In the outside solution there will be a concentration, x, of free Cl ions and free H ions. Hence

$$(x_{H}\text{ outside})\ (x_{Cl}\text{ outside}) =$$

$$(y_{H}\text{ inside})\ (y_{Cl} + z_{Cl}\text{ inside})$$

Since

$$x > y$$

$$C_H \text{ outside} > C_H \text{ inside}$$

or

$$\text{pH outside} < \text{pH inside}$$

In the latter case it is not necessary that the micellar anion be OH ions to account for the higher pH within the suspension. The excess of the acid anion in the micellar solution will force an excess of the free acid out into the external solution. Since the sesquioxides become negative at a pH where all but a small fraction of the acid anions, i.e., Cl, have been displaced by OH ions, it seems evident that the OH ions are, at most, slightly dissociated.

TABLE 65

The pH inside the suspension and in the solution outside a colloidium membrane at equilibrium, of aluminum "silicate" in the negative and positive condition

Suspension number	M.eq. in 25 cc. suspension		Flocculation	μ/sec. 1 volt/cm.	pH outside	pH inside	pH outside minus pH inside	Exchange reaction	
	NaOH	HCl						In 0.5 *N* $BaCl_2$	In 0.5 *N* Na_2SO_4
1	0.08	.....	++++	−1.32	7.70	7.11	0.59	Acid	Acid
2	0.04	.....	++++	−0.76	7.13	6.82	0.31	Acid	Acid
3	0.02	.....	++++	±0.0	6.78	6.51	0.27	Acid	Acid
4	0.01	.....	++++	+0.84	6.56	6.31	0.25	Acid	Alk. (slight)
5	.....	.....	+++	+1.25	6.46	6.20	0.26	Acid	Alkaline
6	.....	0.01	+	+2.02	5.90	5.95	−0.05	Unaffected	Alkaline
7	.....	0.02	+	+2.42	4.90	5.70	−0.80	Alkaline	Alkaline
8	.....	0.04	+	+2.64	4.48	5.61	−1.13	Alkaline	Alkaline

To test this theory an aluminum silicate was prepared as in system 11 (see part III) by mixing 50 millimols $AlCl_3$ with 67.125 millimols Na_2SiO_3 but with only one-tenth the dilution. The supernatant liquid was drawn off and the floc was dialyzed for 30 days to remove the free electrolytes which otherwise would suppress the potential difference and the Donnan distribution. Then 25-cc. portions of the suspension containing 0.0675 gm. Al_2O_3 and 0.079 gm. SiO_2 $\left(\frac{SiO_2}{Al_2O_3} = 1.99\right)$ were adjusted to variouspHvalues by adding NaOH or HCl and were then placed in collodion bags made in large test tubes. These were placed in still larger test tubes, to which water was added (about 20 cc.) to the same level as inside the bags, and allowed to stand 2 weeks. The cataphoresis and the pH measurements were then made, the latter by the quinhydrone electrode. The results are given in table 65.

The negative suspension is more acid than the outside solution but for some reason, difficult to explain, this higher acidity of the suspension extends over on the cataphoretically, positive side. The cataphoretic isoelectric point is

at pH 6.51 and is, as it should be, well within the zone of flocculation. The isoelectric point with respect to the Donnan potential is at a pH about 6.0 since at a pH of 5.95 there is a slightly higher H-ion concentration in the outside solution than in the suspension. Below this pH the suspension is considerably less acid than the outside solution.

This discrepancy in the position of the two isoelectric points has also been observed by Loeb (7) in the proteins: The two points are apparently governed either by somewhat different factors or by the same factors but to a different degree. Why do the particles at a pH of 6.51 show no movement in the electric field and why do they move to the cathode at pH values of 6.31 and 6.20? In all three cases they possess a cation atmosphere. The particles should therefore be negatively charged and migrate to the anode as they do at higher pH values. If the particles are positive as their migration indicates, then how should we account for the cation atmosphere? Is there perhaps, a second outer atmosphere of Cl ions which for some reason, possibly because of a greater hydration, diffuse farther out into the dispersion medium? Then the cation atmosphere is merely a residual inner atmosphere, which nevertheless affects the quinhydrone electrode and also the indicators, because the pH values were checked with the indicator method.

Wiegner and Palmann (15), who have made an extensive and very interesting study of the effect of various colloids in respect to their acid or alkaline dispersion effect, would dispose of the aforementioned difficulty on the basis of their theory that the inner, as well as the outer, ionic layer consists of diffusible ions. The ions in either layer affect the electrode, so that if the colloid is positive, and has therefore an outer anion layer, its dispersion effect will nevertheless be acid as long as the H ions in the inner layer predominate over the OH ions in the outer layer. They do not consider the Donnan equilibrium. To account for the acid or alkaline dispersion effects, they assume the actual preponderance of H or OH ions in the two layers.

Although the author does not deny the adsorption of both of a pair of ions, originally extraneous to the colloid and the formation of a double layer of the ions thus adsorbed, he has here preferred to respresent the colloids as ionogens, dissociating into diffusible and non-diffusible ions since the evidences point overwhelmingly in that direction. This is done without ignoring the possibility of the existence of diffusible ions in the inner as well as in the outer layer. It is, however, difficult to understand why aluminum hydroxide should at a high pH adsorb Cl ions from HCl and be negative and at a low pH adsorb H ions from HCl and become thereby positive, when it can be shown that a combination with Cl ions actually renders the colloid positive.

The observation by Wiegner and Palmann (15, table 20) that the displacement of H ions by NaCl led to a weaker dispersion acidity may be explained on the basis of the suppressing effect of the high salt concentration according to the Donnan equilibrium. It can, of course, be shown that with nothing but Na ions in the micellar atmosphere, we shall, nevertheless, find a higher H-ion concentration inside than outside the micellar solution, because the high Na-ion

concentration within will force an excess of NaOH to the outside. But the Ca-permutite of Wiegner and Palmann, which was electronegative and of an alkaline reaction, produced an alkaline dispersion effect. We are therefore forced to conclude with them, that the micelles contain OH ions in the inner layer, fixed perhaps by "Gitterkräfte, Nebenvalenzattraktion, etc." but free to affect the hydrogen-ion determination. A further study in this direction will undoubtedly give us a better insight into the nature of the electrokinetic phenomena which are among the most difficult of interpretation.

SUMMARY

The cation exchange capacity of isoelectrically precipitated "silicates," "phosphates," and "humates" of aluminum and iron of varying composition ratios has been studied and compared with that of natural soil colloids of varying silica/sesquioxide ratios.

The exchange capacities, at pH 7.0, increase with the proportion of acidoid (SiO_2, P_2O_5, and humus) to ampholytoid (sesquioxides) in all the materials studied. The ferric complexes show a higher cation exchange capacity than the corresponding aluminum combinations.

The differences are explained on the assumption that the acidoid and ampholytoid constituents exist in partial combination with one another, resulting in compounds which are at the same time both acidic and basic. The free acidoid valences, uncombined with the ampholytoid valences constitute the seat of cation exchange.

The exchange capacity is not a constant quantity but can be altered by a shifting of the bonds.

The electronegative complexes give an exchange acidity with neutral salts whereas the electropositive complexes give an exchange alkalinity. The point of exchange neutrality does not, however, coincide with the isoelectric point but varies with the nature of the ions of the salt.

The Donnan equilibrium shows the micellar atmosphere to consist of cations in the electronegative condition and of anions in the electropositive condition but the cataphoretic and the Donnan isoelectric points do not appear at exactly the same pH.

REFERENCES

(1) Anderson, M. S., and Mattson, Sante 1926 Properties of the colloidal soil materials. U. S. Dept. Agr. Bul. 1452.

(2) Breazeale, J. F., and Magistad, O. C. 1928 Base exchange in orthoclase. Univ. Ariz. Tech. Bul. 24.

(3) Burgess, P. S. 1929 The so-called "build-up" and "break-down" of soil zeolites as influenced by reaction. Univ. Ariz. Tech. Bul. 28.

(4) Hendricks, S. B., and Fry, W. H. 1930 Results of X-ray and microscopic examination of soil colloids. *Soil Sci.* 30: 457–479.

(5) Kelly, W. P., Dore, W. H., and Brown, S. M. 1931 The nature of the base exchange material of bentonite, soils and zeolites, as revealed by chemical investigation and X-ray analysis. *Soil Sci.* 31: 25–55.

(6) KERR, H. W. 1928 The identification and composition of the soil alumino-silicate active in base exchange and soil acidity. *Soil Sci.* 26: 385–398.

(7) LOEB, JACQUES 1924 Proteins and the Theory of Colloidal Behavior, ed. 2. New York.

(8) MAGISTAD, O. C. 1929 The use of artificial zeolites in studying base exchange phenomena. *Jour. Amer. Soc. Agron.* 21: 1045–1056.

(9) MATTSON, SANTE 1926 The relation between the electrokinetic behavior and the base exchange capacity of soil colloids. *Jour. Amer. Soc. Agron.* 18: 458–470, 510–512.

(10) MATTSON, SANTE 1927 Anionic and cationic adsorption by soil colloidal materials of varying $SiO_2/Al_2O_3 + Fe_2O_3$ ratio. *Proc. First Internatl. Cong. Soil Sci.* (Washington) 2 (Comn. II): 185–198.

(11) MATTSON, SANTE 1928 The electrokinetic and chemical behavior of the alumino-silicates. *Soil Sci.* 25: 289–311.

(12) MATTSON, SANTE 1929 The laws of soil colloidal behavior: I. *Soil Sci.* 28: 179–220.

(13) PIERRE, W. H., AND SCARSETH, G. D. 1931 The determination of the percentage base saturation of soils and its value in different soils at different pH values. *Soil Sci.* 31: 99–114.

(14) WIEGNER, GEORGE, AND MÜLLER, K. W. 1929 Beiträge zum Ionenumtausch besonders an Permutiten. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* (A) 14: 321–347.

(15) WIEGNER, GEORGE, AND PALLMANN, H. 1929 Über Wasserstoff und Hydroxylschwarmionen um suspendierte Teilchen und dispergierte Ultramikronen. *Verhandl. d. zweiten Komm. u. d. Alkali-Subkomm. d. Internatl. Bodenkundl. Gesell.* (Budapest) B: 92–144.

FELIX LOHNIS

Felix Löhnis

1874–1930

Doctor Felix Löhnis, world famous agricultural bacteriologist, died in Leipzig, Germany, on December 8, 1930. Doctor Löhnis was born in Dresden, Germany, on August 3, 1874, and obtained his academic education in the Universities of Jena, Halle, and Leipzig, obtaining his PhD. from the latter in 1901. After teaching for two years in agricultural schools, he returned to Leipzig and in 1905 was made head of agricultural bacteriology, which position he held until 1914. In this capacity, he was responsible for instruction and research, resulting in the publication of numerous papers on milk and soil bacteriology. A manual of methods, *Landwirtschaftlich-bakteriologisches Praktikum*, made its appearance during this period and was subsequently translated into English, French, Japanese, Polish, and Russian. In 1910 he published the well-known *Handbuch der landwirtschaftlichen Bakteriologie*, the only comprehensive review of the literature in existence at that time. These and two other books, *Einführung in die Bakteriologie* and *Vorlesungen über landwirtschaftliche Bakteriologie*, gained an international reputation for him.

In 1914, a few months before the outbreak of the World War, Dr. Löhnis accepted an appointment in the Office of Soil Bacteriology, U. S. Department of Agriculture, becoming the chief of that office in 1923. Soon after taking up his research in America, he investigated the morphologic changes in cultures of Azotobacter and developed the theory of a definite life cycle, later extending this work to include other bacteria. A review of the literature up to 1918, published as a memoir of the National Academy of Sciences, was done in characteristic Löhnis fashion. Whether one agrees with his views on life cycles or not, it is certain that his work on this subject has exerted a profound influence upon the science of bacteriology, the development of which only the future can tell.

Besides the life cycle work, he carried on field and greenhouse experiments for 10 years on the effect of legumes on succeeding crops, the nitrogen availability of green manures, and allied subjects. During this period, a *Textbook of Agricultural Bacteriology* (with E. B. Fred) made its appearance.

In 1925, Dr. Löhnis resigned as chief of the Office of Soil Bacteriology, the position he had held for two years, and returned to the University of Leipzig as professor and director of the Institute of Agricultural Bacteriology and Soil Science. Students again flocked to his laboratory and in a relatively short time a great amount of research work was undertaken. In addition to the work incident to his teaching and lectures before agricultural groups, he issued a second edition of his *Vorlesungen* in 1926, and in 1929 became editor of the

Centralblatt für Bakteriologie Abt. II. The second edition of the voluminous *Handbuch* was in the course of preparation at the time of his death.

Relatively few Americans knew Löhnis personally. His partial deafness made it unpleasant for him to attend meetings, which he rarely did. His great knowledge of the literature and his ability to recall it made him at once a helpful friend or a severe critic. He was very intolerant of work poorly done, of old facts published as new, or of over-emphasis as to the importance of the work, and he did not hesitate to express himself and in very choice language. Consequently many who came in contact with him only through publication or correspondence knew only that side of his nature; but those who had the privilege of working with him, will long remember a very different personality, jovial, enthusiastic, and always ready to help any investigator who wished to work and showed that he was sincere. Untiring and thorough in his work, whether searching through the literature or looking through a microscope, he was an inspiration to those under his direction; the pity of it is that more persons could not have profited by such an association. His death came as a distinct blow to his former associates, and will mean a great loss to the science of agricultural bacteriology throughout the world.

NATHAN R. SMITH.

CONSERVATION AND AVAILABILITY OF THE NITROGEN IN FARM MANURE

A. FLOYD HECK

University of Wisconsin[1]

Received for publication July 14, 1930

The beneficial effect of animal manure when applied to farm crops has been recognized from very early times. More recent information leads us to believe that this beneficial effect is due, in the main, to the three elements, phosphorus, potassium, and nitrogen, and more especially to the nitrogen, which the manure contains. The value of farm manure has been placed at figures ranging from $2 to $5 a ton, depending on the kind and quality of the manure and the type of farming practice. At these figures, the average dairy cow will produce during the year manure valued at from $26 to $65. Farm manure perhaps has its greatest economic value in the more intensive agricultural districts and to a lesser extent in the districts where dairy farming or general livestock farming is practiced.

During the year, the average dairy cow produces about 13 tons of manure containing 130 pounds of nitrogen, of which about 70 pounds are in the liquid portion. Most of the present investigations indicate that the nitrogen in the liquid manure has the same value as the nitrogen in sulfate of ammonia. At this rate the nitrogen in the liquid manure alone from one cow for one year has a value of about $15. There is grave doubt whether more than half of this nitrogen is utilized on the American dairy farm; in fact there are data to indicate that as much as half of it may be lost. If this is true, the dairy farmers of the state of Wisconsin alone are losing annually over $20,000,000 worth of readily available nitrogen.

Because of the value of the nitrogen in farm manure and the ease with which it is lost, much work has been done on its composition, conservation, and utilization, especially by European workers. Some of this work will be reviewed briefly in relation to the work reported in this paper.

Composition. The nitrogen content of cattle manure varies with several factors, including the kind of animal, kind of feed, season of the year, amount and kind of bedding, and methods of handling the manure. The excrement from cattle is made up of about 70 per cent solid and 30 per cent liquid by weight. The nitrogen content of the dung will vary from 0.20 to 0.45 per cent with an average between 0.35 and 0.40 per cent and the urine from 0.80 or less to over 1.5 per cent. If the complete excrement from the cow is mixed with

[1] From the department of soils. Published with the approval of the director.

enough straw as bedding to absorb the liquid, a complete manure is obtained which contains from 0.50 to 0.55 per cent of nitrogen, half of which is from the liquid manure. If this manure is allowed to stand for a few days the nitrogen from the liquid manure changes to ammonia so that the result is a manure containing 10 to 12 pounds of nitrogen a ton, of which 5 to 6 pounds are in the form of ammonia.

Hansen (15) reports the nitrogen content of stable manure as ranging between 0.5 and 0.6 per cent where the total excrement is included. Of the total nitrogen from 25 to 40 per cent is in the ammonia form. Analyses of manures from the farms in Jutland and Seeland, Denmark (27) show from 0.43 to 0.56 per cent of nitrogen. The Ohio station (2) gives figures to show that the manure, including the litter, from dairy cattle contains 0.57 per cent nitrogen of which 0.285 per cent is water soluble. Analyses of manure from the Hancock (Wisconsin) substation show 0.66 per cent total and 0.18 per cent ammonia nitrogen, and samples from the Marshfield (Wisconsin) substation a total nitrogen content of 0.56 per cent of which not more than 20 per cent is ammonia nitrogen. These figures show that there is less ammonia nitrogen in the samples taken from the farms than should be found if the total excrement is composted with the bedding. This shortage indicates that either there has been a loss of ammonia nitrogen from the manure heap or part of the liquid manure nitrogen is not incorporated with it.

A method, used extensively in Europe, of handling the liquid manure is the use of the manure cisterns. The liquid is separated, as far as possible, from the solid and is stored in underground cisterns. Danish workers (19, 25, 26, 27) have shown that the nitrogen content of the liquid manure from these cisterns varies greatly. On farms where average feeds are used the liquid ranges from 0.45 to 0.55 per cent of nitrogen. If the cisterns are closed tightly there is more nitrogen than where they are more loosely closed or partly open. The nitrogen content varies greatly from the top to the bottom of the tank, ranging from 0.2 per cent at the top to over 0.7 per cent at the bottom. With the exception of from 5 to 10 per cent, this nitrogen is all in the form of ammonia. These workers found that the decrease in nitrogen content from the bottom to the top of the liquid manure is most marked in the surface 2 feet and is due to the loss of ammonia nitrogen by volatilization. This loss is greatest in the poorly covered cisterns. They found that there is no difference in the nitrogen content of the liquid on the same level, whether near the center or near the wall of the cistern. Swiss workers (31) found the nitrogen content of liquid manure in cisterns to range from 0.03 to 0.58 per cent. Five years' analyses of liquid manure gave the average figures of 0.34 per cent total nitrogen and 0.31 per cent ammonia nitrogen. When these data are compared with the fresh urine from cattle containing from 1.0 to 1.3 per cent of nitrogen it seems that there must be a very heavy loss of nitrogen between the animal and the field.

In any manure where the liquid portion is present, the first action to take

place is the change of urea nitrogen to ammonia nitrogen and this change is accompanied by a rise in the pH value. Under normal temperature conditions, this change takes place within about a week. After this there is an acid fermentation similar to that occurring in silage. This fermentation is anaerobic and takes place during a period of from one to two months. During this fermentation carbon dioxide and the volatile organic acids are formed, which in all probability consist largely of acetic and butyric acids. During this fermentation the pH value moves down in proportion to the ratio of fermentable carbonaceous material to ammonia present. Hansen (15, 16) has shown that the pH value of the fermented mixture ranges from 7.0 to 9.0 and the ratio of volatile acids to ammonia varies from 0.85 to 2.53. These biochemical changes leave the liquid manure nitrogen largely in the form of ammonium salts of carbonic and volatile organic acids.

Conservation of manure nitrogen. Aside from mechanical losses, the losses of nitrogen from farm manure come for the most part either in its storage until it is hauled to the field or during the hauling and spreading process and until it is incorporated with the soil.

When the liquid, solid, and bedding are stored together the least loss of nitrogen occurs when the manure is tightly packed and contains all of the water it will hold without drainage. Under these conditions, which are practically anaerobic, manure may be held in containers or manure pits with open tops for long periods with very little loss of nitrogen. Russell and Richards (36) think that this may best be accomplished by use of the "deep stall" and the manure allowed to remain under the animal until it is used. This procedure is not impossible in the dairy industry but makes sanitation more difficult. In some districts a large "deep stall" feeding shed is used where the cows run loose and are placed in the stanchions only for milking. It is doubtful whether this scheme can ever be a practical working plan because of the nature of cattle manure. These English workers report that under anaerobic conditions there is very little loss of nitrogen, but that if the manure is mixed and left somewhat aerobic as much as one-third of the nitrogen may be lost. They seem to think that some of it may be lost as elemental nitrogen. Köhnlein (23) quotes figures to show that only 13 per cent of the nitrogen is lost in 4½ months from the deep stall and that if the manure is removed and piled in a more or less open heap as much as 36 per cent is lost when the pile is covered and 37 per cent when not covered. Danish workers (17, 28) have shown that where both the liquid and solid are stored together with the bedding in bins or storage houses with tight bottoms and the manure is packed solid and wet, the loss of nitrogen may be as low as 2 to 5 per cent of the total. If the conditions are less favorable the loss is greater. Work done at this laboratory with large and small containers indicates that where moist anaerobic conditions are maintained, the loss of nitrogen is very small, and occurs for the most part during the period of ammonification which comes during the first week of storage. After this period, fermentation takes place and the ammonia is held in solution as the salts of volatile acids which are rather stable except on drying.

Storage of liquid. Where the liquid manure is stored separately from the solid it is best done in cisterns or closed containers. European farmers (27, 31) have followed this practice for many years. The cisterns are usually underground and the liquid is either pumped out or the cistern is built on a hillside and emptied by gravity directly into the spreading tank. These reservoirs range from 12 to 20 feet in diameter and from 8 to 10 feet in depth and with a capacity of from 25 to 60 cubic feet for each cow, the size depending on the size of the herd and the length of the storage period. These cisterns are usually built with walls of concrete or masonry and plastered on the inside. The tops are closed and made as nearly air tight as possible.

Since the nitrogen in stored liquid manure is largely in the form of ammonium carbonate it is easily lost. Jensen (21, 22) has shown that there is a vapor pressure to an ammonia solution directly proportional to the alkalinity. He has also shown that this is likewise true of the ammonium salts of the volatile acids. These facts help to explain the reports of Kristensen (25, 26) that the nitrogen in the liquid manure decreases from the bottom toward the top of the liquid, and that the top 2 or 3 feet may contain as little as 0.2 per cent of nitrogen, because of the volatilization of the nitrogen as ammonia from the liquid. If the ammonia can be changed to a salt of one of the more stable acids, such as the nitrate, sulfate, chloride, or phosphate, then there is no vapor pressure of ammonia and hence it is not lost.

To prevent the loss of nitrogen from the liquid manure, different means have been tried with varied success. Attempts have been made to use disinfectants (11, 12, 35) in order to slow up the ammonification process and thus slow up the losses of ammonia nitrogen. Success from this measure has been small. Kainite and gypsum have also been used in this rôle with only meager success. Ammonification may be slowed up by low temperature (9, 12) but the control of temperature is impracticable. Chemical treatment (12, 22, 23, 29) offers a means to prevent volatilization of ammonia nitrogen by the use of such substances as calcium sulfate, calcium chloride, or calcium nitrate, when the calcium is precipitated as chalk and the ammonia is held as the less volatile sulfate, chloride, or nitrate. Soluble phosphates such as superphosphate or treble superphosphate may also be used in the same way. One of the strong mineral acids (10, 11), such as sulfuric or phosphoric will also hold the nitrogen in a less volatile form. Although these treatments are more or less effective, either their cost of some other factor makes them impracticable. Danish workers (19, 27) found that the more tightly the cisterns are closed the less is the loss of ammonia nitrogen by volatilization and that the cistern must be sealed air tight to prevent the loss entirely. A layer of oil (8, 9, 35) over the surface of the liquid manure has been found to reduce materially the losses from volatilization. Blanck (9) also found that dilution of the liquid manure reduced the loss of ammonia nitrogen. This is no doubt due to the reduction of vapor pressure of the ammonium carbonate.

The losses of nitrogen from handling manure. If fresh manure is hauled

directly to the field from the animal before any ammonification of the urea takes place there is less loss of nitrogen because neither the urea or dung nitrogen is volatilized before ammonification. If, on the other hand, the manure has gone through the ammonification period or has passed on through the fermentation process, the urea or liquid manure nitrogen has changed to the ammonium salts of carbonic or volatile organic acids and will be lost by volatilization when the damp manure is exposed to drying. Hansen (15, 16) and Iversen (20) report that when manure is spread out on zinc plates it loses water, ammonia nitrogen, and volatile acids. At 25°C. it loses 75 per cent of its weight and 75 per cent of its ammonia nitrogen in four days. In the open the loss is greatest in clear, warm spring weather with wind, and this amounts to 25 per cent of the total nitrogen in four days. Availability experiments conducted at the Danish stations indicate that a loss of one-half of the ammonia nitrogen may occur from complete fermented manure on being spread and exposed during favorable drying weather for 24 hours before its incorporation with the soil.

There are also losses from handling the liquid manure. Liechti and Ritter (30), found that when the liquid is spread on the soil by sprinkling there is a loss of from 4 or 5 per cent to as much as 20 per cent of the total nitrogen. They found little advantage where superphosphate is used. Houcamp and Blanck (18) found that, when incorporated with the soil, urine and liquid manure give about the same availability of nitrogen as calcium nitrate, sodium nitrate, and ammonium sulfate, but when top-dressed, the returns are less. If the urine or liquid manure is treated with sulfuric or phosphoric acid it gives the same yield whether top-dressed or plowed under. Blanck (9) found that the dilution of liquid manure materially reduces the losses of ammonia nitrogen by volatilization. He (10) also showed that when the untreated liquid manure is applied to the surface of a sandy soil there are heavy losses of nitrogen. The higher the temperature and the older the liquid manure, the greater are these losses. Less of the nitrogen is lost from the liquid manure when applied beneath the surface or mixed immediately with the soil. Machines (24, 38) for this purpose are used by European farmers with cultivated crops in the intensive farming districts but are not so practical on the extensive American farm.

Liquid manure as such, is little used on American farms, generally on account of their extensive nature. Teutsch (39), however, reports the use of liquid manure on pasture lands in the dairy district of western Oregon.

Availability of manure nitrogen. Figures showing the availability of the nitrogen in farm manure vary greatly, depending much upon several factors such as quality and relation of soluble to insoluble nitrogen, method of handling, amounts of manure applied, and the crop grown. Hansen (17) reports that where field applications of farm manure are made at the rate of 9,000 kgm. per hectare (about 4 tons per acre) from 37.6 to 43.6 per cent of the nitrogen is available. If one-half this quantity is applied, the availability is from 48.4 to

57.8 per cent, but if one and one-half times this quantity is used, the availability drops to 33 per cent of the total nitrogen applied. At the same time he found that mineral nitrogen gives an availability of 56.0 to 64.5 per cent of the nitrogen applied. Iversen (20), reporting results from two of the Danish stations, shows that at Lyngby for the years 1910–1921, it required 2.33 units of manure nitrogen to equal 1 unit of inorganic nitrogen and at Askov the requirement was 1.9 units of manure nitrogen to equal 1 unit of inorganic nitrogen. These figures indicate that over half of the nitrogen in good farm manure is not available for crop growth. This is borne out by the work of Barthel and Bengtsson (3, 6, 7) in which they show that it is only the ammonia nitrogen, in the main from the liquid manure, that is available to the growing crop. These workers maintain that it is by the conservation and use of the liquid manure that the greatest returns are obtained and that the value of the manure depends very much on this factor. The means of storage and the fermentation through which it goes determine to some extent the value of the manure and the availability of its nitrogen. Scheibe (37) found that manure stored and fermented in closed containers suffers less loss, decomposes more readily in the soil, and the nitrogen is better utilized by crops. Goeters (13) reports that a 3 month's fermentation increases the availability of the nitrogen from 6.6 per cent to 18.4 per cent.

The availability of the nitrogen in farm manure at English and American experiment stations varies greatly and falls generally below that reported by Danish workers. Hall (14) reports the recovery of only 30 per cent of manure nitrogen by mangels, whereas from mineral nitrogen and rape cake the recovery was from 60 to 70 per cent. There was an accumulation of nitrogen in the plot where manure was applied. Apparently the effect of this increase in soil nitrogen is seen in the barley yields on Hoose field for a period of 40 years after the application of manure had been discontinued.

From the New Jersey station Lipman and Blair (32) report 20 years' work on the availability of nitrogen in farm manure and other fertilizers. This work was done in tanks under controlled conditions and showed only 32.7 per cent of the nitrogen in manure available as against 62.4 per cent from sodium nitrate. Later work from this station (33) reports field work showing the following availability of cow and horse manure compared with sodium nitrate for four consecutive 5-year periods:

	PER CENT OF AVAILABILITY			
	First 5 years	Second 5 years	Third 5 years	Fourth 5 years
16 T. cow manure—no lime	14.9	14.6	18.7	21.2
16 T. horse manure—no lime	14.6	16.2	24.6	....
160 lbs. $NaNO_3$—no lime	23.5	16.3	16.3	25.1
16 T. cow manure—lime	13.6	12.8	13.2	9.9
16 T. horse manure—lime	11.9	10.9	14.8	....
160 lbs. $NaNO_3$—lime	37.6	39.1	15.7	32.7

Analyses of the soil at the end of the 20-year period show that a large part of the nitrogen not recovered from the farm manure is found accumulated in the soil, but there is no accumulation of nitrogen in the plots treated with sodium nitrate. The effect of lime on the availability of manure nitrogen at New Jersey is similar to the findings of Barthel and Bengtsson (4, 5) who report no beneficial effects from either quicklime or limestone, and that large amounts of lime are inhibitory.

The returns from the use of manure nitrogen at the Ohio Agricultural Experiment Station (34) are rather indefinite but interesting nevertheless. By applying to the crop yields average figures for their nitrogen contents, the amount of nitrogen recovered in the crop is obtained, and by comparing the manured plots with the plots receiving approximately the same amount of phosphorus and potassium contained in the manure, an approximate availability per cent for the manure nitrogen is obtained. During a 15-year period on a 5-year rotation of corn, oats, wheat, and two years of hay at Strongville, 8 tons of farm manure shows no recovery of nitrogen from the manure, either on the limed or float treated land. At Wooster on a 5-year rotation of corn, oats, wheat, clover, and timothy, 8 tons of manure applied once in the rotation shows no recovery of the manure nitrogen during a 29-year period. Double this amount of manure gives a 33 per cent recovery of the nitrogen. On a 4-year rotation of corn, oats, wheat, and clover at Wooster, 4 tons of manure an acre for each rotation gives no recovery, but 8 tons an acre shows a recovery of 10 to 12 per cent of its nitrogen over a period of 34 years. From the very nature of the methods used to obtain these figures, it can be easily seen that they are not absolute; in fact they are somewhat contrary to ordinary experience and also to the findings of Danish workers, who report the greatest percentage returns from the lowest per acre application of manure. Although the significance of these figures is doubtful, when taken together with other availability figures there is an indication that the availability of the nitrogen in American farm manure is much lower than is generally thought, no doubt because of the poor storage and handling methods and also its low ammonia content.

GREENHOUSE WORK ON THE AVAILABILITY OF MANURE NITROGEN

Two sets of pot tests were conducted in the greenhouse to determine the availability of the nitrogen in farm manure. One set of these tests was conducted with synthetic cow manure and horse manure made by mixing with straw the solid and liquid excrement in a definite proportion approximating that voided by the animal. The other test was made on dairy manure from the storage heap at the Marshfield substation. In the former case the start was made with fresh manure, and definite records were made on the storage and drying losses as well as the amount recovered in the four crops which were grown on the same pots over a period of two years.

This work was done in 2-gallon glazed earthenware pots. The soil was a

type intermediate between a sandy loam and a loamy sand very low in organic matter and nitrogen. All pots were supplied with sufficient lime, phosphorus, and potassium so as to eliminate all of these as factors. Each pot contained

TABLE 1

Kinds of manure and amounts of nitrogen added

	POT NUMBER	TREATMENT	NITROGEN ADDED PER POT (10 KGM. SOIL)			
			Dung	Urine	Straw	Total
			mgm.	*mgm.*	*mgm.*	*mgm.*
Controls	1–4		...	...	...	...
Cow manure, fresh	5–6	Dung only	311.7	...	...	311.7
	7–8	Dung and urine	311.7	345.6	...	657.3
	9–10	Dung, urine, and straw	311.7	345.6	74.9	732.2
Cow manure, fresh, dried	11–12	Dung only	290.2	...	...	290.2
	13–14	Dung and urine	290.2	179.2	...	469.4
	15–16	Dung, urine, and straw	290.2	249.8	74.9	614.9
Cow manure, fermented	17–18	Dung only	289.4	...	...	289.4
	19–20	Dung and urine	289.4	298.8	...	588.2
	21–22	Dung, urine, and straw	289.4	287.2	74.9	651.5
Cow manure, fermented, dried	23–24	Dung only	287.7	...	...	287.7
	25–26	Dung and urine	287.7	46.4	...	334.1
	27–28	Dung, urine, and straw	287.7	74.3	74.9	436.9
Cow manure, frozen dry	29–30	Dung only	275.7	...	...	275.7
	31–32	Dung and urine	275.7	259.3	...	535.0
	33–34	Dung, urine, and straw	275.7	314.6	74.9	665.2
Horse manure, fresh	35–36	Dung only	335.7	...	...	335.7
	37–38	Dung and urine	335.7	299.3	...	635.0
	39–40	Dung, urine, and straw	335.7	299.3	74.9	709.9
Horse manure, fresh, dried	41–42	Dung only	317.5	...	...	317.5
	43–44	Dung and urine	317.5	194.0	...	511.5
	45–46	Dung, urine, and straw	317.5	177.2	74.9	569.6
Horse manure, fermented	47–48	Low straw, top	...	...	...	530.4
	49–50	Low straw, bottom	...	...	...	652.7
	51–52	High straw, top	...	...	...	516.0
	53–54	High straw, bottom	...	...	...	535.2
Horse manure, fermented, dried	55–56	Low straw, top	...	...	...	528.8
	57–58	Low straw, bottom	...	...	...	360.6
	59–60	High straw, top	...	...	...	538.0
	61–62	High straw, bottom	...	...	...	336.0

10 kgm. of soil. The manure was applied at the rate of 12 tons an acre, 120 gm. a pot on the basis of the complete fresh manure. The manure was made up of 74 per cent by weight of dung, 20 per cent of urine, and 6 per cent of chopped straw. This was also true of the horse manure used, except that marked "high straw," in which 18 per cent of straw was used. Where the dung alone or dung and urine were added without straw, 74 per cent and 94 per cent respectively of the 120 gm. were added. If there were losses in storage or drying, this was deducted from the 120 gm. so that the application was always made on the fresh wet basis except for the fermented horse manure. Table 1 gives the treatments of the manures and the amounts of nitrogen added to the various pots.

The fermentation took place in 2-gallon stoneware containers at a temperature of 20°C. for four weeks. The tops of these containers were loosely covered to prevent excessive evaporation. The drying was done by spreading 500-gm. portions of the wet manure on glass plates in the greenhouse and allowing it to dry at 20°C. Complete air-drying took place in about five to six days. The manure was then removed from the plates and dried in the oven at 80°C. over night.

The manure frozen dry was spread on a glass plate and held at a temperature below freezing until dry. This required about two weeks. It was then dried in the oven.

Analyses were made both on the wet and dry samples. Wet analyses were made on 20-gm. samples in triplicate. After digestion, the samples were made up to 500 cc. and two-fifths of the sample was used. This procedure gave excellent results. It was found that the total nitrogen contained in manure could not be obtained after the samples were dried because of the loss in drying.

The manure was thoroughly incorporated with the moist soil by mixing and sifting. The plants were watered with distilled water. Four crops—oats, buckwheat, corn, and oats—were grown in rapid succession with no further additions of manure and with only from one to three months intervening between the crops. Table 2 gives yields and nitrogen content of those four crops. Each of these figures is the average of duplicate cultures.

None of these crops were grown to maturity but only to the point where they had used all of the available nitrogen. This came in the oats at about the heading stage, with the buckwheat when it was in blossom and with the corn when the first tassel appeared. The crops were then cut off at the surface of the soil and the roots left in the pot. The tops were oven-dried, weighed, ground, and the nitrogen determined. By deducting the nitrogen in the controls from that obtained from the other pots, the amount of nitrogen recovered from the manure is obtained. Table 3 shows these figures, together with the total amounts of nitrogen obtained in the four crops and the amounts added in the manure.

TABLE 2

Yields of dry matter and nitrogen contents of the four crops: oats, buckwheat, corn and oats

Amounts per pot (10 kgm. soil)

	POT NUMBER	OATS, FIRST CROP		BUCKWHEAT, SECOND CROP		CORN, THIRD CROP		OATS, FOURTH CROP	
		Yield	Nitrogen	Yield	Nitrogen	Yield	Nitrogen	Yield	Nitrogen
		gm.	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*
Controls (average)	1–4	7.60	0.98	5.1	1.19	9.65	0.98	3.45	1.44
Cow manure, fresh	5–6	7.50	1.15	6.6	1.15	10.65	0.95	4.25	1.46
	7–8	25.40	1.38	5.0	1.25	14.90	1.05	3.80	1.60
	9–10	22.50	1.35	4.1	1.29	19.40	0.95	3.75	1.68
Cow manure, fresh, dried	11–12	5.80	1.41	8.5	1.11	11.30	0.96	4.60	1.50
	13–14	14.40	1.15	4.3	1.39	13.15	0.90	4.35	1.59
	15–16	13.10	1.21	3.7	1.38	14.15	0.85	4.35	1.76
Cow manure, fermented	17–18	8.00	1.22	8.3	1.20	14.75	0.90	4.90	1.53
	19–20	20.70	1.34	4.4	1.42	17.15	0.89	3.85	1.58
	21–22	19.60	1.26	6.6	1.24	12.95	1.03	5.75	1.46
Cow manure, fermented, dried	23–24	6.00	1.38	6.5	1.11	12.15	0.84	4.15	1.48
	25–26	6.70	1.22	5.5	1.16	10.80	0.94	4.85	1.44
	27–28	6.90	1.37	7.6	1.08	9.90	0.96	5.10	1.45
Cow manure, frozen dry	29–30	5.40	1.37	7.7	1.06	10.55	0.98	4.70	1.39
	31–32	16.90	1.31	4.6	1.27	12.65	1.14	4.65	1.35
	33–34	17.30	1.29	3.6	1.30	13.25	1.03	4.95	1.43
Horse manure, fresh	35–36	3.85	1.85	8.5	1.12	13.85	0.95	4.95	1.29
	37–38	20.00	1.34	4.1	1.44	16.00	1.04	5.40	1.28
	39–40	18.25	1.28	5.6	1.34	21.00	0.94	4.70	1.38
Horse manure, fresh, dried	41–42	2.95	1.96	8.6	1.04	14.55	0.79	5.15	1.27
	43–44	8.45	1.18	5.3	1.23	15.70	0.87	4.65	1.47
	45–46	6.40	1.47	8.4	1.06	15.55	0.90	5.80	1.35
Horse manure, fermented	47–48	8.05	1.33	8.8	1.16	15.30	0.95	5.30	1.32
	49–50	18.85	1.29	5.1	1.33	10.50	1.05	5.10	1.36
	51–52	9.95	1.24	7.7	1.16	16.45	0.86	5.20	1.31
	53–54	14.85	1.20	4.8	1.24	16.45	0.87	4.85	1.32
Horse manure, fermented, dried	55–56	5.95	1.47	7.4	1.19	12.50	0.83	6.00	1.23
	57–58	3.15	2.07	7.4	1.10	14.35	0.84	4.70	1.29
	59–60	8.35	1.13	4.4	1.32	15.95	0.87	4.95	1.36
	61–62	2.10	2.04	7.5	1.10	13.85	0.83	5.40	1.17

TABLE 3

Nitrogen added in the manure and that recovered in the four crops: Oats, buckwheat, corn, and oats

Amounts per pot (10 kgm. soil)

	POT NUMBER	NITROGEN ADDED		NITROGEN RECOVERED FROM MANURE BY FOUR CROPS				
		Urine	Total	Oats	Buck-wheat	Corn	Oats	Total
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Cow manure, fresh	5–6	...	311.7	11.7	15.2	6.7	20.0	53.6
	7–8	345.6	657.3	276.0	1.8	61.9	11.0	350.7
	9–10	345.6	732.2	229.2	−2.8	70.8	13.3	305.5
Cow manure, fresh, dried	11–12	...	290.2	7.3	33.7	14.0	19.3	74.3
	13–14	179.2	469.4	91.1	0.1	23.8	19.3	134.3
	15–16	249.8	614.9	84.0	−9.6	26.8	26.8	128.0
Cow manure, fermented	17–18	...	289.4	23.1	38.9	38.2	25.3	125.5
	19–20	298.8	588.2	202.9	1.8	58.1	11.3	274.1
	21–22	287.2	651.5	172.5	21.1	38.9	34.5	267.0
Cow manure, fermented, dried	23–24	...	287.7	8.3	11.4	10.5	11.8	42.0
	25–26	46.4	334.1	7.2	3.1	7.0	20.0	37.3
	27–28	74.3	436.9	20.5	21.4	0.5	24.2	66.6
Cow manure, frozen dry	29–30	...	275.5	−0.5	21.9	9.0	15.7	46.1
	31–32	259.3	535.0	146.9	−2.3	49.7	13.1	207.4
	33–34	314.6	665.2	148.7	−13.9	43.3	21.3	199.4
Horse manure, fresh	35–36	...	335.7	−3.3	34.5	37.0	14.5	82.7
	37–38	299.3	635.0	194.5	−1.6	72.0	19.4	284.3
	39–40	299.3	709.9	159.1	14.4	103.8	15.3	292.6
Horse manure, fresh, dried	41–42	...	317.5	−16.8	28.9	20.4	15.8	48.3
	43–44	194.0	511.5	25.2	4.5	32.8	18.5	81.0
	45–46	177.2	569.6	19.6	28.4	45.4	28.7	122.1
Horse manure, fermented	47–48	Not known exactly	530.4	34.2	41.4	50.8	20.1	146.5
	49–50		652.8	169.6	6.1	15.7	19.7	211.1
	51–52		516.0	48.9	20.5	46.9	18.7	135.0
	53–54		535.2	103.8	−1.1	48.5	14.3	165.5
Horse manure, fermented, dried	55–56	Not known exactly	528.8	13.0	22.5	9.2	24.0	68.7
	57–58		360.6	−9.3	20.7	26.0	10.8	48.2
	59–60		538.0	19.9	2.6	45.0	17.9	80.2
	61–62		336.0	−31.7	21.8	20.4	13.4	23.9

Minus means less than the control.

A balance sheet showing the amounts of nitrogen lost in the various storing and handling operations is given in table 4. This table also shows the percentage recoveries from the various treatments based both on the original total and also on the original amount of liquid manure nitrogen present.

Losses of nitrogen in the handling of manure occur at two points; first, during the process of storage, and second, in the process of spreading and exposure before the manure is incorporated with the soil. The figures in table 4 show a fermentation loss of 7 to 11 per cent. This will vary directly with the exposure, temperature, and evaporation. If the manure is in a deep, moist heap and well packed, only slightly exposed at the top and with no drainage, the loss from storage will not be more than a few per cent, which comes largely during the ammonification period. These data correspond to the findings of other investigators already cited.

The second and perhaps greatest loss of the nitrogen from farm manure comes during the drying period from the time the manure is spread in the field until it is plowed under. These handling, spreading, and drying losses vary greatly with the conditions, being greater with the higher temperature and more excessive drying. The losses given in table 4 for spreading and drying would be the maximum that could possibly occur. The loss of nitrogen from the dung by drying is always small, whatever the previous treatment may have been. This would be expected because practically all of the dung nitrogen is organic and only about 20 per cent of it is changed to ammonia in storage.

The greatest loss of nitrogen comes from the urine or liquid manure. When the liquid is mixed with the dung only and the fresh material allowed to dry, there is a loss of 28.6 per cent of the total nitrogen, or about twice that percentage of the liquid manure nitrogen. This loss comes largely because the manure does not dry fast enough to prevent ammonification and its consequent loss. The low temperature of the frozen manure gives a lesser loss, due perhaps for the most part to the lack of ammonification. The drying losses are much greater in the fermented manure because the liquid manure nitrogen is entirely changed to ammonia and held in solution as salts of volatile acids. The figures show a loss under these conditions of from 30 to 45 per cent of the total nitrogen, which is approximately from about 50 to 90 per cent of the liquid manure nitrogen. One very outstanding fact in connection with the fermented horse manure is the difference in the drying loss between the top and bottom of the heap. From the manure in the top 5 or 6 inches of the heap there is little or no loss of nitrogen on drying, but when the manure from the bottom portion of the heap is dried, from 80 to 90 per cent of its liquid manure nitrogen is lost by volatilization. This is explained by the fact that the liquid manure nitrogen in the bottom of the heap (strictly anaerobic fermentation) is entirely in the form of ammonium salts of volatile acids and is lost easily, whereas the nitrogen in the top part has passed through an aerobic decomposition, has lost some of its ammonia during the process and the rest has been changed to an organic form by microörganisms, and is not volatile on drying.

TABLE 4

Losses and recovery of nitrogen by the four crops from 120 gm. of manure or its component parts in the various treatments

Amounts per pot (10 kgm. soil)

	POT NUMBER	ORIGINAL NITROGEN IN MANURE		NITROGEN LOST IN STORAGE (FERMENTATION)		NITROGEN LOST IN HANDLING (DRYING)		NITROGEN RECOVERED IN 4 CROPS			NITROGEN LOST PLUS THAT RECOVERED IN 4 CROPS	
		Urine	Total					Original total		Original urine		
		mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*per cent*	*mgm.*	*per cent*
Cow manure, fresh	5–6	...	311.7	...	...	...	...	53.6	17.2	...	53.6	17.2
	7–8	345.6	657.3	...	...	...	...	350.7	54.3	101.6	350.7	54.3
	9–10	345.6	732.2	...	...	...	...	305.5	41.7	88.5	305.5	41.7
Cow manure, fresh, dried	11–12	...	311.7	...	...	21.5	6.9	74.3	23.8	...	95.8	30.7
	13–14	345.6	657.3	...	...	187.9	28.6	134.3	20.4	38.9	322.2	49.0
	15–16	345.6	732.2	...	...	117.3	16.0	128.0	17.5	37.1	245.3	31.5
Cow manure, fermented	17–18	...	311.7	22.3	7.16	...	...	125.5	40.3	...	147.8	47.5
	19–20	345.6	657.3	69.1	10.5	...	...	274.1	41.7	79.4	343.2	52.2
	21–22	345.6	732.2	80.7	11.2	...	...	267.0	36.5	77.3	347.7	47.7
Cow manure, fermented, dried	23–24	...	311.7	22.3	7.16	1.7	0.54	42.0	13.5	...	76.0	21.2
	25–26	345.6	657.3	69.1	10.5	254.1	38.7	37.3	5.6	10.8	360.5	54.8
	27–28	345.6	732.2	80.7	11.2	214.6	29.3	66.6	9.1	19.3	361.9	49.6
Cow manure, frozen dry	29–30	...	311.7	...	...	36.0	11.5	46.1	14.8	...	82.1	26.3
	31–32	345.6	657.3	...	...	121.4	18.5	207.4	31.5	60.0	328.8	50.0
	33–34	345.6	732.2	...	...	67.0	9.1	199.4	27.2	52.7	266.4	36.3
Horse manure, fresh	35–36	...	335.7	...	...	...	...	82.7	24.6	...	83.7	24.6
	37–38	299.3	635.0	...	...	...	...	284.3	44.8	95.0	284.3	44.8
	39–40	299.3	709.9	...	...	...	...	292.6	41.2	97.7	292.6	41.2
Horse manure, fresh, dried	41–42	...	335.7	...	...	18.2	5.4	48.3	14.4	...	66.5	19.8
	43–44	299.3	635.0	...	...	123.5	19.4	81.0	12.7	27.0	204.5	32.1
	45–46	299.3	709.9	...	...	140.3	19.8	122.1	17.2	40.8	263.4	37.0
Horse manure, fermented	47–48	Not	530.4	...	...	...	...	146.5	27.6	...	146.5	27.6
	49–50	known	652.8	...	...	...	...	211.1	31.1	...	211.1	31.1
	51–52	exactly	516.0	...	...	...	...	135.0	26.2	...	135.0	26.2
	53–54		535.2	...	...	...	...	165.5	30.9	...	165.5	30 9
Horse manure, fermented, dried	55–56	Not	530.4	...	...	1.6	0.3	68.7	13.0	...	70.3	13.3
	57–58	known	652.8	...	...	292.2	44.8	48.2	7.4	...	340.4	52.2
	59–60	exactly	516.0	...	...	No loss		80.2	15.6	...	80.2	15.6
	61–62		535.2	...	...	199.2	37.2	23.9	4.5	...	223.1	41.7

Straw as bedding in the manure tends to reduce the drying losses and the more straw used, the greater is the saving of nitrogen. Although straw shows up as an aid in preventing loss at this point, it is a decided detriment to the availability of the nitrogen, which is inversely proportional to the amount of straw present. In this case the straw furnishes energy material for the growth of soil organisms, especially fungi, which compete with the plant in the use of nitrogen and cause a lower recovery of nitrogen in the crop. Allison (1) and many other workers have shown that the energy materials supplied in manure have this effect.

The recovery of the manure nitrogen by the four crops varied from almost nothing to over 50 per cent of the total nitrogen or approximately 100 per cent of the liquid manure nitrogen. The availability of the dung nitrogen is low

TABLE 5

Analysis of stored solid and liquid manure from the Marshfield and Hancock (Wisconsin) substations

	COMPLETE MANURE STORED IN THE HEAP	LIQUID MANURE FROM THE CISTERN
Marshfield, 1928:		
Dry matter, per cent	23.3	...
Specific gravity	...	1.0134
Total nitrogen, per cent (wet)	0.56	0.33
Total nitrogen, per cent (dry)	1.97	...
Nitrogen lost on drying, per cent (wet)	0.10	...
Total phosphorus, per cent (wet)	0.115	...
Total potassium, per cent (wet)	...	0.426
Hancock, 1929:		
Total nitrogen, per cent (wet)	0.665	0.35
Ammonia nitrogen, per cent (wet)	0.183	0.33
Potassium, per cent (wet)	...	0.336

except in case of the fermented dung, where it reaches 40 per cent of the nitrogen, but in most cases is below 20 per cent. With the cow manure, where the dung and liquid alone are used, the amount of nitrogen recovered in the crop is the difference between the total liquid manure nitrogen and the losses occurring before the manure is incorporated into the soil. If two-thirds of the liquid manure nitrogen is lost in storage and handling, only one-third is recovered in the crops. The availability of the nitrogen in horse manure is usually a little lower than that from cow manure; first, because there is usually a lesser proportion of liquid manure nitrogen present, and, second, there is more energy material of a cellulose nature. Straw nearly always lowers the availability in direct proportion to the amount present. Manure decomposed aerobically and well rotted shows a lower availability than the anaerobically fermented manure because of the change from ammonia to organic nitrogen in the aerobic decomposition.

Tests with Manure from the Marshfield Substation. Manures from the storage heap and liquid manure cistern at the Marshfield substation were also used in pot tests. Table 5 shows the composition of these manures from Marshfield for 1928 and also from the Hancock substation for 1929.

The composition of these manures is very different from that of the synthetic manures used in the previous tests. Although the total nitrogen in the manure

TABLE 6

Application and recovery of nitrogen from manure from the Marshfield substation applied to fallow pots

Amounts per pot (10 kgm. soil)

POT NUMBER	TREATMENT	MANURE APPLIED	NITROGEN ADDED IN MANURE	NITRATE NITROGEN RECOVERED FROM FALLOW		
				Total	From original manure	
		grams	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	Control, no manure	...	...	130	...	...
2	Manure fresh from heap—turned under immediately	120	672	340	210	31 3
3	Manure fresh from heap—wet down 3 or 4 times, allowed to dry, then turned under	120	672	310	180	26 8
4	Manure dried before application	28 (dry)	552	160	30	5.6
5	Liquid manure, turned under immediately	30 4	100	240	110	110.0

TABLE 7

Recovery of nitrogen by corn from manure from the Marshfield substation

Amounts per pot (10 kgm. soil)

POT NUMBER	WEIGHT OF DRY MATERIAL			NITROGEN IN DRY MATERIAL					NITROGEN ADDED IN MANURE	RECOVERY OF ORIGINAL MANURE NITROGEN ADDED	
	Tops	Roots	Total	Tops		Roots		Total			
	grams	*grams*	*grams*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	10.5	7.5	18 0	0.77	80.8	0.92	69 0	148 8	...	...	...
2	25.3	9.7	35.0	0.97	245.4	1.01	98.0	343.4	672	194.6	28.9
3	22.6	11 0	33.6	0.86	194.4	0 93	102 3	296 7	672	147.9	22.0
4	15.5	10.3	25.8	0.73	113.1	0.86	88.6	201.7	552	52.9	9.5
5	19.4	10.5	29.9	0.80	155.2	0 86	90.3	245.5	100	96.5	96.5

is high, as much as 13 pounds of nitrogen to a ton of manure, the ammonia portion is very low. This condition may be due to the failure to incorporate all of the liquid into the storage heap or its subsequent loss by leaching, or it may be due to a more or less aerobic condition of the heap and a change from ammonia to organic nitrogen or even some losses of ammonia nitrogen.

Pot tests with the manure from Marshfield were made as already outlined.

The complete manure was applied at the rate of 12 tons an acre and the liquid manure 3 tons an acre. Four pots of each treatment were made, two of which were kept in clean fallow and two planted to corn. The corn was grown to the tasseling stage, harvested, the roots removed from the soil, and both top and roots were dried, weighed, and analyzed. The fallow pots were carried along until the harvest of the corn when the amount of nitrate nitrogen was determined in them. Table 6 shows the treatments, amounts of nitrogen applied to all pots, and the nitrogen recovered as nitrate from the fallow pots. Table 7 gives the yields of corn, its nitrogen content, and the nitrogen recovered from the manure by the corn.

It is interesting to note that the amounts of nitrogen recovered in the corn are approximately the same as those obtained as nitrate from the manure in the fallowed pots. In this case, moisture was not a factor and it would be logical to expect as much nitrification in one case as in the other. With this particular manure, less than 30 per cent of its total nitrogen is recovered in the total crop when the manure is incorporated in the soil at once with no loss of nitrogen. This indicates that between 30 and 50 per cent of the liquid manure nitrogen either was not incorporated into the storage heap or was lost by leaching or volatilization in the storage process. When the manure is wet down after spreading, in imitation of rain, and a part of the soluble nitrogen washed into the soil before the manure is allowed to dry prior to plowing down, there is a recovery of less than 25 per cent of the total nitrogen, thus showing an additional loss from the drying after the rain of 5 to 7 per cent of the total nitrogen. If the manure is dried completely before application, the availability is reduced to almost nothing, showing that practically all of the ammonia nitrogen is lost when the manure dried at 80°C. Figures in table 10 show that 95 per cent of the ammonia nitrogen from fermented manure is lost by drying in this way and this corresponds closely to the availability to corn of this manure nitrogen.

When the liquid manure is used alone its nitrogen is practically all recovered in the crop. This nitrogen is completely water-soluble and largely in the ammonia form with no energy material present. This liquid manure, however, was very low in nitrogen, carrying only 10 pounds a ton. To apply 30 pounds of nitrogen to the acre in this form it would be necessary to apply 3 tons, or about 750 gallons, of the liquid to the acre.

STORAGE AND HANDLING CHANGES IN MANURE; LABORATORY ANALYSES

Changes in storage. A series of laboratory studies were made to determine the nature of the changes taking place in manure when stored and how these changes affect the losses in handling. A series of closed containers were set up and filled with urine, dung, and mixtures of these, together with straw. The mixtures were made up on the basis of dung 74 per cent, urine 20 per cent, and straw 6 per cent for the complete manure. The tops of these containers were closed and the air space above the manure filled with carbon dioxide more

nearly to imitate anaerobic conditions in the compact heap. A mercury seal was placed on each container. These containers were held at 20°C. for 30 days and then determinations were made for total and ammonia nitrogen, volatile and non-volatile acids, and pH. Table 8 gives these data.

The urine nitrogen changes almost completely to ammonia, only about 5 per cent remaining in organic form. Because of the large amount of ammonia formed, the pH value goes up to 9.4 at the end of 30 days. This change is complete in a short time with practically no subsequent change.

TABLE 8

The composition and reaction of urine, dung, and cow manure mixtures on standing

	URINE ONLY	DUNG ONLY	DUNG AND URINE	COMPLETE MANURE
			Dung: 78.7% Urine: 21.3%	Dung: 74% Urine: 20% Straw: 6%
Nitrogen in urine, per cent	1.106	.....	1.106	1.106
Nitrogen in dung, per cent	.....	0.386	0.386	0.386
Nitrogen in straw, per cent	.....	.....	......	1.04
Urine nitrogen in mixture, per cent	1.106	.....	0.2356	0.2212
Dung nitrogen in mixture, per cent	.....	0.386	0.3038	0.2856
Straw nitrogen in mixture, per cent	.....	.....	......	0.0624
Total nitrogen in mixture at beginning, per cent	1.106	0.386	0.5394	0.5692
After 30 days:				
Total nitrogen, per cent	1.077	0.402	0.525	0.575
Ammonia nitrogen, per cent	1.018	0.084	0.278	0.271
Volatile acids per gram of manure, cc. $N/14$ acid	.....	2.20	2.772	4.295
Non-volatile acids per gram of manure, cc. $N/14$ acid	.....	1.12	0.784	0.9075
pH	9.4	5.27	7.37	6.79
After 60 days:				
Total nitrogen, per cent	.....	.....	......	0.574
Ammonia nitrogen, per cent	.....	.....	......	0.296

In 30 days only about 20 per cent of the dung nitrogen is changed to ammonia. This material is acid at the end of this period with a pH value of 5.27 and volatile acids equivalent to 2.2 cc. of $N/14$ acid per gram of manure, or nearly three times the amount of acid necessary to unite with the ammonia nitrogen present. This is the most acid fermentation because it contains no urine and consequently little ammonia nitrogen is produced to raise the pH value. After 30 days a little over half of the nitrogen in the dung and urine mixture is ammonia. The volatile acids in this mixture are equivalent to 2.77

cc. of $N/14$ acid per gram of manure, a little increase over the dung alone, but the pH value is up to 7.37 because of the large amount of ammonia present. When straw is added to the dung and urine there is an increase in the amount of volatile acids and a drop in the pH value. The addition of straw has practically no effect upon the ammonification process or the amount of ammonia produced, but the energy material in the straw materially increases the amount of volatile acid produced. At 60 days there is a slight increase in the ammonia content.

Ammonia traps were placed inside of each of these containers and, with the exception of the liquid alone, there is practically no volatilization of ammonia nitrogen. It seems that loss of nitrogen as ammonia from manure mixtures is always accompanied by evaporation.

TABLE 9

Changes in the nitrogen content of fermented manure exposed to drying

Amounts from 20-gm. sample

	AMMONIA NITROGEN	NON-AMMONIA NITROGEN	
		Water-soluble	Water-insoluble
	mgm.	*mgm.*	*mgm.*
Atmosphere still—no wind:			
12 hours—20°C	50.2	13.0	47.8
36 hours—20°C	32.0	10.7	43.6
3½ days—20°C	21.4	11.65	39.8
7 days—20°C	17.0	10.4	41.6
7 to 8 days*—80°C	3.0	8.1	45.4
Atmosphere moving—8½-mile wind:			
12 hours—20°C	30.0	11.8	40.8
36 hours—20°C	23.1	12.1	40.3
3½ days—20°C	17.7	12.6	40.6
7 days—20°C	15.7	11.4	40.4
7 to 8 days*—80°C	2.85	8.5	43.4
In manure before exposure	59.2	57.5	

* See note, table 10.

Losses of manure nitrogen in handling. A study was made of the losses of nitrogen from the complete manure mixture of dung, urine, and straw shown in table 8. Samples of a given weight were spread out on glass plates at the rate of 12 tons an acre. They were allowed to stand at room temperature (20°C.) and humidity. One set was exposed to a still atmosphere and another set to air moving at the rate of 8½ miles an hour. They were not exposed to sunshine. At varying intervals of time, the ammonia nitrogen and non-ammonia nitrogen were determined. The last samples, after standing at room temperature for 7 days, were heated at 80°C. for 24 hours. The ammonia

was determined by distilling the water-soluble fraction of the manure with magnesium oxide. These data are given in table 9.

There is very little change in the non-ammonia nitrogen but the ammonia nitrogen is almost completely lost under the conditions of the experiment. Table 10 gives the losses of ammonia nitrogen in per cent based both on the total and the ammonia nitrogen. From these data it may be seen that in seven days nearly three-fourths of the ammonia nitrogen in fermented manure is lost by volatilization. When the atmosphere is in motion, the loss is much more rapid in the beginning, but at the end of a 7-day period, approximately the same amounts are lost. Heating to 80°C. causes a further loss of about 20 per cent and leaves only a 5 per cent residue of ammonia nitrogen. The 8½-mile wind causes a greater loss in 12 hours than is lost from the samples dried without wind in 36 hours. Almost one-half of the ammonia nitrogen is lost in 12 hours with the 8½-mile wind. The loss of nitrogen from the drying

TABLE 10

Losses of ammonia-nitrogen from fermented manure exposed to drying

Amounts from 20-gm. sample

AFTER	ATMOSPHERE STILL—NO WIND			ATMOSPHERE MOVING—8½ MILE WIND		
	Total nitrogen		Ammonia nitrogen	Total nitrogen		Ammonia nitrogen
	mgm.	*per cent*	*per cent*	*mgm.*	*per cent*	*per cent*
12 hours—20°C.	9.0	7.7	15.2	29.2	25.1	49.4
36 hours—20°C.	27.2	23.4	46.0	36.1	30.9	61.0
3½ days—20°C.	37.8	32.4	63.9	41.5	35.6	70.0
7 days—20°C.	42.2	36.2	71.3	43.5	37.3	73.5
7 to 8 days*—80°C.	56.2	48.2	95.0	56.4	48.3	95.2

* After air-drying in the open for 7 days the residue was placed in the 80° oven for 24 hours.

fermented manure seems to take place in two stages. In the first stage the loss is very rapid and uniform and this stage covers approximately one-half of the ammonia nitrogen. In the second stage the loss is much slower and represents about one-half of the remaining ammonia nitrogen.

A further study of the volatile substances given off from manure was made in an attempt to explain the reason for the volatilization of the ammonia nitrogen. A small container 10 inches by 2 inches by ½ inch deep was arranged so that 30 gm. of manure could be spread on the bottom at the rate of 12 tons an acre and then carbon dioxide- and ammonia-free air was drawn over the manure and the ammonia, carbon dioxide, and volatile acids were trapped and determined. This was carried on for 144 hours in three 48-hour periods. The results are given in table 11.

These data, together with those in table 10, show that the loss of ammonia nitrogen proceeds up to the extent of about 50 per cent largely as the carbonate,

with perhaps little loss as the salts of volatile acids. This loss, taking place while the manure is still moist, is very rapid and follows almost a straight line. When the moisture is gone, the loss of nitrogen is for the most part ammonium salts of the volatile acids and is much slower. These volatile acids are in all

TABLE 11

Ammonia nitrogen, carbon-dioxide carbon, and volatile acids lost in 144 hours from drying fermened manure

Amounts from 30-gm. sample of complete manure

	AMMONIA NITROGEN	CARBON-DIOXIDE CARBON	VOLATILE ACIDS
	mgm.	*mgm.*	*cc. N/14 acid*
First 48 hours	51.8	38.4	10.5
Second 48 hours	11.2	6.0	10.5
Third 48 hours	6.8	7.8	4.2
Total volatilized in 144 hours	69.8	52.2	25.2
Total ammonia nitrogen in sample.			
Total nitrogen in sample			172.2

TABLE 12

Losses of nitrogen from liquid manure under different methods of storage

	OPEN—NO TREATMENT	OPEN—50 GM. 45 PER CENT SUPERPHOSPHATE	OPEN—½ INCH OIL COVER
At beginning:			
Amount used, cc.	700	700	680
Specific gravity, liquid	1.03	1.03	1.03
Total nitrogen, per cent	1.29	1.29	1.29
At end of 30 days:			
Loss in weight, gm.	40	32	0
Ammonia nitrogen, per cent	0.952	0.998	1.242
Total nitrogen, per cent	1.039	1.159	1.277
Weight liquid manure, gm.	681	689	700.4
Weight of nitrogen in manure, gm.	6.94	7.98	8.99
Loss of nitrogen, gm.	2.38	1.34	0.07
Loss of nitrogen, per cent	25.5	14.37	1.08
At end of 6 months:			
Loss of nitrogen, per cent	....	....	1.27

probability largely acetic and butyric. These acids were isolated as a group and gave a Duclaux curve similar to the lower organic acids, but no definite identifications were made.

Storage losses from liquid manure. Three flasks of fresh urine were set up in the laboratory and held at 20°C. They were left unstoppered. No. 1 was

held as a control with no treatment, no. 2 received 50 gm. of 45 per cent superphosphate, and no. 3 received no chemical treatment but was covered with a layer of lubricating oil ½ inch deep. They were allowed to stand for 30 days and analyses made of their contents. Table 12 shows the results of this study.

The control lost 25.5 per cent of its nitrogen in 30 days; the phosphate-treated urine, 14.4 per cent; and the oil-covered urine, only 1 per cent in 30 days and only 1.27 per cent after a period of 6 months. If urine is treated with a mineral acid or a soluble salt of a mineral acid, especially a calcium salt, the ammonia in the liquid manure is changed to the salt of a non-volatile acid and is not lost from the solution. None of these chemical treatments have proved practical for farm use, either because of the expense or some other disadvantage, such as the insoluble residue left by the superphosphate, which must be removed fom the cistern in the solid form.

The layer of oil, however, is a very efficient and cheap means of conserving the liquid manure nitrogen. From a practical standpoint, used crank case oil can be obtained at a very low cost and the saving of nitrogen is almost 100 per cent. The delivery pipe and the pump can be run to the bottom of the cistern and the liquid manure delivered and pumped out from under this oil layer, which forms a perfect seal. Losses of nitrogen on spreading may be reduced by dilution before spreading or by drilling the liquid under the surface of the soil.

DISCUSSION

Farm manure may be applied to the land as soon as it is produced or it may be stored until some convenient time in the cropping system. Either method may be used with advantage at times, and if handled properly, with a minimum loss of nitrogen, but perhaps by far the greater portion of farm manure is stored before application. For general livestock or dairy farming this is probably the simplest and best adapted. Although European farmers have used the separate storage to advantage in their more intensive farming, it is a question whether it will ever come into general use in the more extensive American system. It seems that for the more extensive farmer the storage of the dung, urine, and bedding together is most convenient. This storage should accomplish three things; namely, the incorporation of all of the liquid excrement with the manure, the prevention of any leaching, and the maintenance of anaerobic conditions in the storage heap. The first is accomplished by the use of gutters for the prevention of loss, and the use of sufficient absorbent or bedding. Leaching in storage is best prevented by the use of some sort of concrete container with a tight bottom so that no liquid may be lost. It is not necessary for this manure container to be covered, in fact unless the rainfall is very high it is better for the manure to receive the normal precipitation, as long as there is no leaching. This precipitation insures the necessary moisture to produce and maintain anaerobic conditions and these conditions are better produced by packing the normally moist manure than by water-logging. There is

enough moisture in cattle manure so that if it is packed down, anaerobic conditions will result. European farmers are careful of this point and often use animals for the packing. This stored manure quickly ammonifies and then in the course of a month or two, depending upon the temperature, goes through a fermentation process similar to that of silage fermentation. If this storage is done properly the end product will contain practically all of the nitrogen in the original dung and urine, and about half of it, or that portion originally in the urine, will be in the form of ammonium carbonate and the ammonium salts of volatile organic acids. If this process is properly done, the loss of nitrogen in storage of manure should be less than 10 per cent of the original nitrogen.

The handling and spreading of anaerobically stored manure must be done properly if minimum losses are to be obtained. Three-eights to one-half of the nitrogen in this manure is easily lost by exposure to drying conditions. Greatest spreading losses occur when weather conditions are best for drying. Warm, dry, sunny days with wind produce conditions which cause greatest losses. If the spreading is done during cool, rainy weather and the manure incorporated with the soil immediately, the losses may be reduced to a minimum. The handling of fermented manure is made much easier if cut straw is used for liter. The spreading of fresh manure is attended by the loss of less nitrogen than where the manure is fermented. The practice of applying the fresh manure to snow-covered fields in winter gives low handling losses, but on the other hand, if fresh manure is spread and allowed to lie on the land during warm sunny weather, ammonification takes place within two or three days and the losses may be heavy.

The available nitrogen in farm manure is largely confined to the ammonia or water-soluble portions. The water-soluble nitrogen in farm manure, minus the losses in storage and handling, gives a very good measure of the nitrogen in manure which is readily obtained by the growing crop. Very little of the dung nitrogen is readily utilized by crops. Only in the case of fermented dung does any appreciable amount of the dung nitrogen become available. The amount of the nitrogen in the liquid furnishes a very good measure of the value of the nitrogen in farm manure. When straw is used for bedding to absorb the liquid, it not only acts as an absorbent, but also aids in decreasing the losses on drying after the manure is spread. Although the straw is an advantage in this respect, it is a decided disadvantage in the amount of manure nitrogen recovered in the crop. The straw furnishes energy for the growth of soil microorganisms with a resultant depression in the nitrate and available nitrogen. As a result, the greater amount of straw used as bedding, the greater the depression in the availability of the manure nitrogen. This depression is especially marked in the first crop following the manure application.

SUMMARY

This paper reports a laboratory and greenhouse study of the changes, losses, and availability of the nitrogen in farm manure. The results may be summarized as follows:

When manure is stored under anaerobic conditions two changes take place; namely, the urea and water-soluble nitrogen change to ammonia, and an acid fermentation takes place with the formation of volatile organic acids which react with the ammonia, forming salts.

The losses of nitrogen from farm manure come both in the storage and handling processes. Under extreme conditions these losses combined may be almost one-half of the total nitrogen or nearly the whole of the liquid manure nitrogen. The loss of nitrogen is due to the volatilization of ammonia either as the carbonate or as the salt of a volatile acid. The former seems to be lost most rapidly during the early drying period and the latter when most of the carbonate is gone. The ammonification process results in an increase in the pH value and the fermentation process causes the pH value to decrease, and this decrease is in direct proportion to the amount of straw or energy material present.

When liquid manure is stored alone, the nitrogen changes to ammonium carbonate and is lost from the solution by volatilization. This loss may be reduced to almost nothing by covering the surface of the liquid with a thin layer of mineral oil. Although the container may be open, there is practically no loss of nitrogen through the oil layer.

Straw as bedding in the manure tends to reduce the drying losses of nitrogen, but at the same time it reduces the availability of manure nitrogen.

An approximate measure of the nitrogen available from farm manure is obtained by the difference between the water-soluble or ammonia nitrogen and the losses of nitrogen from the manure during storage and handling. If there is no loss of nitrogen from cattle manure, 50 per cent of the total, or 100 per cent of the liquid, manure nitrogen should be recovered in the first crop under normal conditions. A smaller recovery than this is probably due either to loss of liquid manure nitrogen or the use of excessive amounts of straw as bedding.

REFERENCES

(1) Allison, F. E. 1927 Nitrate assimilation by soil microörganisms in relation to available energy supply. *Soil Sci.* 24: 79–93.

(2) Ames, J. W., and Gaither, E. W. 1912 Barnyard manure. Ohio Agr. Exp. Sta. Bul. 246.

(3) Barthel, C. 1926 Neure Untersuchungen über die Ausnützung des Stallmiststickstoffes im Ackerboden. *Fortschr. Landw.* 1: 37–41.

(4) Barthel, C., and Bengtsson, N. 1918 Bidrag till frågan om stallgödselkvävets nitrifikation i åkerjorden. II. Meddel. Centralanst. Försöksv. Jordbruksområdet. (Sweden) 172.

(5) Barthel, C., and Bengtsson, N. 1920 Bidrag till frågan om stallgödselkvävets nitrifikation i åkerjorden. III. Meddel. Centralanst. Försöksv. Jordbruksområdet. (Sweden) 211.

(6) Barthel, C., and Bengtsson, N. 1924 Bidrag till frågan om stallgödselkvävets nitrifikation i åkerjorden. IV. Meddel. Centralanst. Försöksv. Jordbruksområdet. (Sweden) 269.

(7) Barthel, C., and Bengtsson, N. 1926 Bidrag till frågan om stallgödselkvävets nitrifikation i åkerjorden. V. Meddel. Centralanst. Försöksv. Jordbruksområdet. (Sweden) 311.

(8) Berendt, E. Mechanische Konservierung des organischen Düngers. J. Neumann, Neudamm.

(9) Blanck, E. 1918 Studien über den Stickstoffhaushalt der Jauche. Teil I. Über die Umwandlung und den Verlust des Stickstoffs in Harn und Jauche. *Landw. Vers. Sta.* 91: 173–221.

(10) Blanck, E. 1918 Studien über den Stickstoffhaushalt der Jauche. Teil 3. Über den Stickstoffverlust von Jauche und Harn bei der Auf- und Einbringung bezw. Kopfdüngung. *Landw. Ver. Sta.* 91: 271–290.

(11) Blanck, E. 1918 Studien über den Stickstoffhaushalt der Jauche. Teil 4. Der Einfluss des Formalins und der Schwefelsäure auf die Konservierung und Düngewirkung des Jauche-Stickstoffs. *Landw. Ver. Sta.* 91: 309–346.

(12) GERLACH, PROF. DR. 1919 Über die Konservierung, den Düngewert und die Verwendung der Jauche. *Landw. Jahrb.* 53: 77–107.

(13) GOETERS, W. 1929 Untersuchungen über Keimgehalt und Wirkung verschieden behandelten Stalldüngers mit besonderer Berücksichtigung des nach dem Verfahren von H. Krantz bereiteten Eidelmistes. *Landw. Vers. Sta.* 108: 1–60.

(14) HALL, A. D. 1917 The Book of the Rothamsted Experiments. New York.

(15) HANSEN, FRODE 1927 Staldgødningsundersøgelser. I. *Tidsskr. Planteavl.* 33: 753–780.

(16) HANSEN, FRODE 1929 Staldgødningsundersøgelser. II. *Tidsskr. Planteavl.* 35: 81–110.

(17) HANSEN, N. A. 1928 Gødningsforsøg paa Forsøgsstationen ved Aarslev 1911–1926. *Tidsskr. Planteavl.* 34: 373–523.

(18) HOUCAMP, F., AND BLANCK, E. 1916 Über die Konservierung und Düngewirkung des Jauchestickstoffes. *Arb. Deut. Landw. Gesell.* 282: 40–78.

(19) IVERSEN, K. 1925 Undersøgelser vedrørende Ajlens Opbevaring. 1924. *Tidsskr. Planteavl.* 31: 149–168.

(20) IVERSEN, K. 1927 Gødningsforsøg paa Forsøgsstationerne ved Askov og Lyngby. *Tidsskr. Planteavl.* 33: 557–752.

(21) JENSEN, S. T. 1928 Undersøgelser over Ammoniakfordampning i Forbindelse med Kvaelstoftab ved Udbringning af naturlige Gødninger. I Ajle. *Tidsskr. Planteavl.* 34: 117–147.

(22) JENSEN, S. T. 1929 Undersøgelser over Ammoniakfordampning i Forbindelse med Kvaelstoftab ved Udbringning af naturlige Gødninger. II. Ajle. *Tidsskr. Planteavl.* 35: 59–80.

(23) KÖHNLEIN, J. 1926 Stalldüngerkonservierung. *Fortschr. Landw.* 1: 386–388.

(24) KRAFT, G. 1919 Die Ackerbaulehre. Paul Parey, Berlin.

(25) KRISTENSEN, R. K. 1906 Undersøgelser over opbevaret Ajles Kvaelstofindhold i forskellige Partier af Ajlekummen og over forskellige Fremgangsmaader ved Udtagning af Ajleprøver til kemiske Analyse samt nogle Iagttagelser og Forsøg over Forhold vedrørende Ajlens Opbevaring in Kummerne. *Tidsskr. Planteavl.* 13: 235–250.

(26) KRISTENSEN, R. K. 1907 Undersøgelser af Ajle saerlig med Hensyn til dens Tab af Kvaelstof under Opbevaringen. *Tidsskr. Planteavl.* 14: 276–291.

(27) KRISTENSEN, R. K. 1907 Undersøgelser af fast Staldgødning og Ajle fra Gaarde i Jylland og paa Sjaelland. *Tidsskr. Planteavl.* 14: 515–570.

(28) KRISTENSEN, R. K. 1910 Opbevaringsforsøg med Staldgødning Forberedende Forsøg. *Tidsskr. Planteavl.* 17: 1–78.

(29) KRISTENSEN, R. K. 1919 Konservering af Ajle. *Tidsskr. Planteavl.* 26: 485–490.

(30) LIECHTI, P., AND RITTER, E. 1913 Ueber das Entweichen von Ammoniak aus begülltem Boden. *Landw. Jahrb. Schweiz.* 27: 429–451.

(31) LIECHTI, P., AND TRUNINGER, E. 1913 Zur Frage des Gehaltes der Gülle an Pflanzennährstoffen. *Landw. Jahrb. Schweiz.* 27: 452–467.

(32) LIPMAN, J. G., AND BLAIR, A. W. 1918 Twenty years' work on the availability of nitrogen in nitrate of soda, ammonium sulfate, dried blood, and farm manures. *Soil Sci.* 5: 291–301.

(33) LIPMAN, J. G., BLAIR, A. W., AND PRINCE, A. L. 1928 Field experiments on the availability of nitrogenous fertilizers. 1923–1927. *Soil Sci.* 26: 1–25.

(34) Maintenance of soil fertility, The. 1924 Ohio Agr. Exp. Sta. Bul. 381.

(35) ORTMANN, C. 1913 Die Schependorfer Jauche-Untersuchungen und einige sich daran anknüpfende Bemerkungen. *Fühling's Landw. Ztg.* 62: 367–373.

(36) RUSSELL, E. J., AND RICHARDS, E. H. 1917 The changes taking place during the storage of farmyard manure. *Jour. Agr. Sci.* 8: 495–563.

(37) Scheibe, K. 1929 Untersuchungen über Abbau und Wirkung der im Stalldünger enthaltenen Stickstoff- und Kohlenstoff-Verbindungen. *Landw. Vers. Sta.* 108: 61–114.

(38) Schipf. 1918 Schipf's populäres Handbuch der Landwirtschaft. Paul Parey, Berlin.

(39) Teutsch, W. L. 1929 Liquid manure used in Oregon. *Hoard's Dairyman* 74: 502.

PLATE 1

Growth of Oats on Soils Variously Treated

Fig. 1. Comparative growth of oats on the control and also on the dung alone from cow manure treated in various ways.

Fig. 2. Comparative growth of oats on fresh cow manure and the same after being dried (Pots 5 and 11 are dung alone; 7 and 13 are dung and urine; and 9 and 15 are dung, urine, and straw).

Fig. 3. Comparative growth of oats on fermented cow manure and the same after being dried (Pots 17 and 23 are dung alone; 19 and 25 dung and urine; and 21 and 27 dung, urine, and straw).

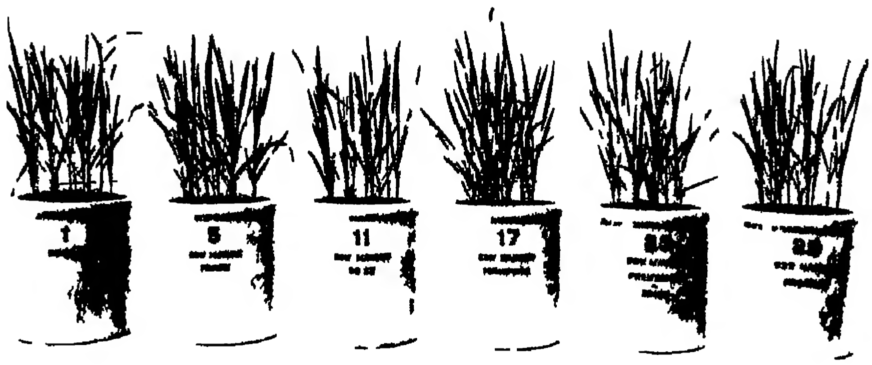


Fig 1

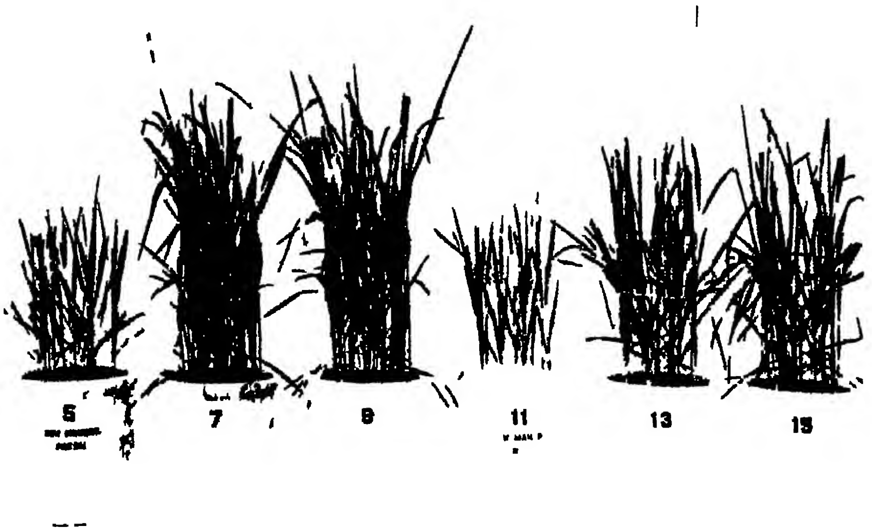


Fig 2

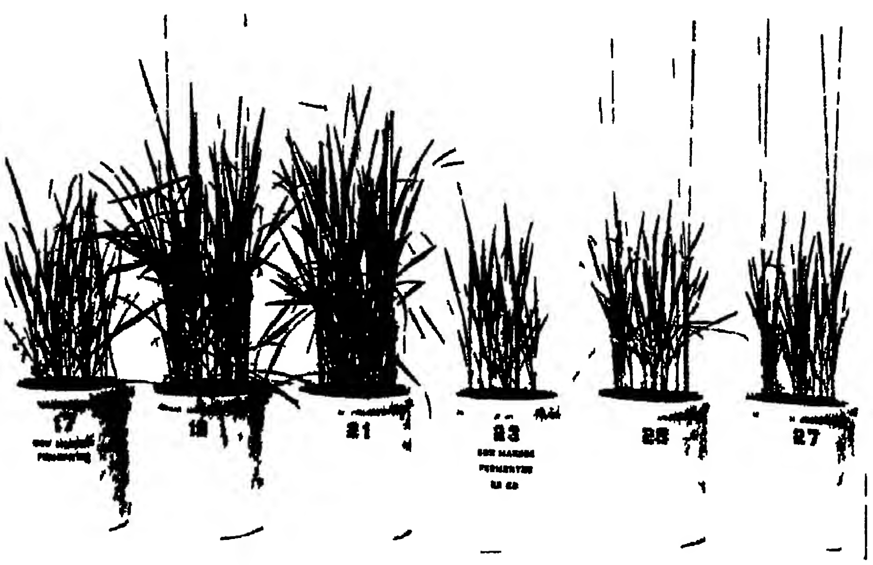


Fig 3

PLATE 2

GROWTH OF BUCKWHEAT ON SOILS VARIOUSLY TREATED

FIG. 1. Comparative growth of buckwheat following oats on the same pots shown in plate 1, figure 2.

FIG. 2. Comparative growth of buckwheat following oats on the same pots shown in plate 1, figure 3.

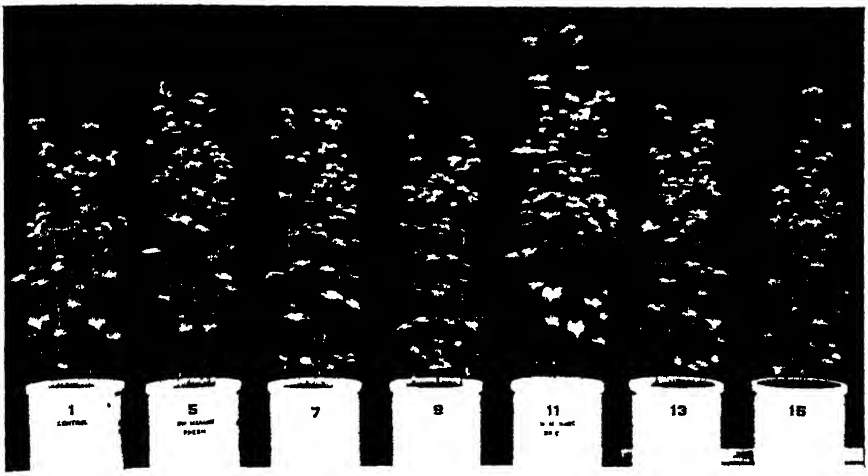


FIG. 1

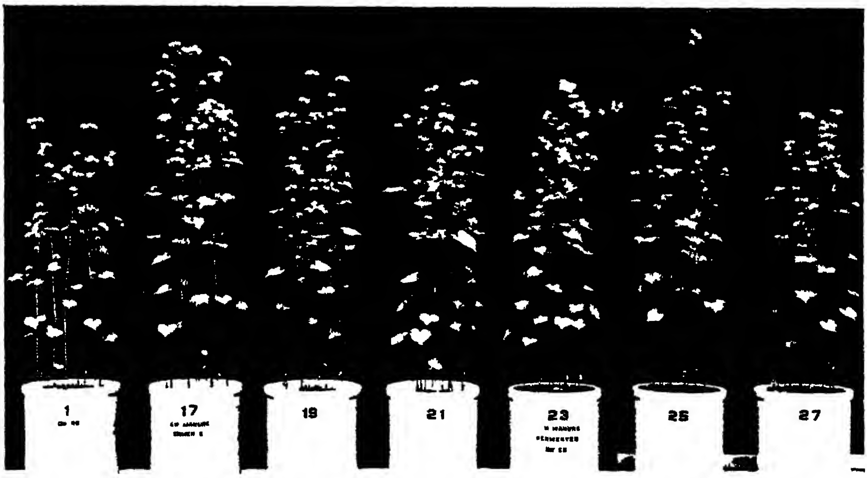


FIG. 2

THE INTERRELATIONSHIPS OF CERTAIN SINGLE-VALUED SOIL PROPERTIES[1]

G. B. BODMAN AND ESTHER P. PERRY

University of California

Received for publication July 28, 1930

It is the purpose of this paper to present the results of some single-valued measurements of the members of a group of soils, to compare the values obtained for the different kinds of measurements, and to discuss briefly the utility and probable significance of the determinations carried out.[2]

The chief object of making a "single-valued measurement" of a soil, as this term is now understood, is to obtain an expression which characterizes the physical behavior of the soil in some one or more ways, and which in addition may provide a useful and simple numerical value indicative of its texture. Several such measurements have been proposed and utilized from time to time, some of them with much success. In this country the hygroscopic coefficient and moisture equivalent have been used more extensively than any others. More recent single valued measurements which have been used here and elsewhere include the colloid content, the clay content, the shrinkage, the pore space, the sticky point, the stiffness, and the Atterberg values. The single-valued measurements which have been used in this work are as follows:

- Colloid content by adsorption,
- Content of clay finer than 2μ effective diameter,
- Content of clay finer than 1μ effective diameter (determined by two different methods of pretreatment),
- Air-dry moisture content,
- Moisture equivalent,
- Sticky point,
- Rolling-out limit.

Fifty-seven soils were used. Detailed descriptions of 44 of these have already been given elsewhere (4). The same numbers have been retained for them, so that they may be identified readily with respect to grouping and origin. The 13 additional soils, numbered 51 to 54, 57, 59, and 61 to 67, comprise seven additional series from the San Joaquin Valley and adjacent foothills in California. They have been included for the purpose of increasing the geographical

[1] Paper read before the meeting of the Western Society of Soil Science at Corvallis, Oregon, June 17, 1930.

[2] The authors wish to thank Dr. Sylvia L. Parker for her criticism of the statistical portion of the paper.

distribution and broadening the already wide textural range. The entire group represents a wide variety in respect to age, origin, climate, and vegetation. All of the soils have been preserved in glass jars for at least 1 year, and in some cases for as long as 15 years, and were air-dry. Each was prepared before use by being passed through a 1-mm. screen, followed by thorough mixing.

COLLOID BY ADSORPTION AND CLAY CONTENT

The influence of the colloid portion of the soil mass upon the physical, physico-chemical, and chemical properties of the whole is recognized. Various methods have been devised for measuring this part of the soil. Some involve indirect measurements in which the extent to which soils manifest certain properties characteristic of the colloid portion alone is measured. Others are more nearly direct, in that some arbitrary particle size limit is set as representing the approximate upper limit of size of the colloidal material, and separations or analyses based on these size limits are then used. The work of Anderson (3), Gile (7), and others has produced evidence favoring the use of 1μ as the arbitrary upper limit of diameter for soil colloid, and according to Gile (8) this is the upper limit implied in the definition of soil colloid by the U. S. Department of Agriculture, Bureau of Chemistry and Soils. In a recent publication, however, Olmstead (11, p. 12) names the material below 2μ effective diameter the "colloid" fraction. Colloid chemists usually consider a much smaller diameter as representing the upper limit of the colloidal state.

One indirect and two direct methods of measurement were used in the present case. W. O. Robinson's (13) procedure was followed in the determination of the water vapor adsorbed per gram of soil. The soils, previously sifted through a 1-mm. brass sieve, were exposed over 3.3 per cent sulfuric acid at 30°C. in a partially evacuated glass desiccator, stored for 5 days in a dark air thermostat. By dividing the water vapor adsorbed per gram of soil by 0.3 (13, 4) the percentage of colloid by adsorption was calculated.[3] The pipette method, following pretreatment with hydrogen peroxide and hydrochloric acid, washing, and shaking with dilute ammonia, was also used to measure the amounts of dispersible material having as upper limits of diameter 1μ and 2μ respectively. This pretreatment is part of the international method as adopted by the International Society of Soil Science (5). It aims at complete dispersion. A modified form of the aforementioned method, very similar to that adopted by the International Society of Soil Science for technical and petrographical purposes and which includes dispersion by shaking with dilute ammonia water only followed by pipette sampling, is a method that has been used to some extent as a rapid means of estimating the readily dispersible clay content of soil samples. This was used as a third method in the present series of measurements.

[3] The measurements of colloid by adsorption were made in connection with (4) by M. Tamachi.

Where both the 2 and 1μ samples were taken from the same suspension following the peroxide and acid pretreatment, the method followed was to sample for the material finer than 2μ first, the pipette being carefully lowered into, and withdrawn from the suspension. The cylinder was then left standing for the additional length of time necessary to allow for the settling of particles 1μ in diameter before again sampling with the pipette. In the international grouping of particle sizes, an effective diameter of 2μ is the upper limit of clay.

A comparison of the results obtained by these three methods, and a consideration of the yield of clay having a maximum particle diameter of 2μ, are of interest. The results are given in table 1 for 44 soils. In 34 of the 44 soils, or in 77 per cent of the total, the highest quantity of colloid is calculated to be present when the water-vapor adsorption method is used, and comparisons are made with the results of the pipette methods of analysis, in which latter the quantities of material of 1μ in diameter and smaller are taken as measures of the colloid content. Furthermore, in 31 of the 44 soils, or in 70 per cent of the total, the colloid by adsorption exceeds the yield of material finer than 2μ obtained by the international method. In all cases but one (soil 6) the lowest yield of colloid is obtained when dispersion is produced by shaking with ammonia water only. Somewhat similar observations have been made by Gile (7) and by Joseph (9).

The results do not simplify the problem of the determination of the quantity of colloid in soils. They do indicate, however, on the one hand that pipette sedimentation determinations following the international method may provide misleading information through failing to take into account the possible presence of colloid coatings still adhering to the surfaces of the larger particles, or of micropores within the particles. Such particles might contribute considerably to the adsorptive capacity of the soil, without undergoing much reduction in settling velocity on account of lowered density. On the other hand the presence of soluble salts in the soil, and the nature of the replaceable bases (16) may slightly increase the quantity of adsorbed water vapor, and consequently the amount of colloid when calculated by the adsorption method. It is planned to study shortly the effect of the former possibility. It is clear that pretreatment by shaking with dilute ammonia water consistently gives figures considerably below those representing either the total dispersible colloid or the total quantity of water vapor-adsorptive material. Such incomplete dispersion obtained by simple shaking in ammonia water is of common occurrence. It has been explained by Wiegner (19).

Upon comparison of the quantities of material yielded by the international method of pretreatment and analysis, and having as upper limit of diameter 1 and 2μ respectively, it is found that for the 44 soils whose content of clay finer than 2μ ranged from 16 to 60 per cent, the average yield of clay of 1μ diameter and less, amounted to 89 per cent of the 2μ material for each soil. A range of from 36 to 100 per cent was obtained for this relationship. However, the lower values are rare, as only 4 of the 44 soils yielded an amount of material

Colloid and clay content as obtained by different methods of analysis, and air-dry moisture content, of 44 soils

SOIL NUMBER	COLLOID BY ADSORPTION	CLAY 1*	CLAY 2†	CLAY 3‡	RATIO: CLAY 1 / CLAY 2	AIR DRY MOISTURE CONTENT
	per cent	*per cent*	*per cent*	*per cent*		*per cent*
41	13.8	15.7	18.6	10.9	0.844	1.0
42	18.5	24.4	26.6	10.2	0.917	2.1
24	19.8	17.2	18.1	9.7	0.949	2.3
44	21.7	25.4	27.6	9.0	0.920	2.0
45	21.7	21.1	25.0	13.9	0.844	1.9
43	24.0	22.1	23.5	13.4	0.940	2.6
40	27.2	23.4	25.5	13.5	0.917	4.0
6	28.7	25.2	25.4	25.6	0.992	6.2
10	30.8	13.5	16.6	6.0	0.813	4.7
18	31.9	15.2	23.8	13.2	0.638	3.7
25	32.7	25.8	29.8	23.5	0.866	3.6
33	33.4	14.7	20.0	8.6	0.735	4.7
49	35.1	23.5	28.6	7.5	0.821	4.3
20	36.8	27.6	32.0	17.4	0.862	3.1
50	36.8	45.8	45.4	9.3	1.008	5.5
7	38.0	53.5	56.0	29.0	0.955	11.5
5	38.8	31.4	32.6	12.3	0.975	5.4
11	40.9	30.2	32.0	13.4	0.943	6.1
22	41.9	18.7	21.8	8.8	0.857	6.4
16	43.6	33.9	36.5	14.1	0.928	5.6
26	45.3	46.0	48.0	26.6	0.958	6.0
12	45.6	45.4	46.3	24.3	0.981	6.8
32	48.8	50.1	53.3	28.1	0.940	6.9
48	49.4	15.7	36.8	14.1	0.426	6.0
47	49.6	13.4	36.9	8.4	0.363	6.9
31	49.7	47.4	49.4	28.5	0.959	6.8
29	50.0	49.9	51.6	26.2	0.968	6.5
3	50.1	35.8	37.3	14.6	0.959	7.7
8	50.2	36.1	38.3	20.6	0.942	6.0
30	50.5	52.4	53.3	26.0	0.983	6.7
15	50.8	32.2	39.9	15.1	0.807	6.1
28	52.5	58.9	58.7	27.0	1.003	7.9
9	54.3	60.0	61.8	25.6	0.970	8.1
27	55.0	45.0	46.9	25.1	0.959	5.5
34	55.7	48.5	55.7	21.9	0.870	9.3
14	56.2	52.5	51.9	25.4	1.012	7.7
13	56.6	38.3	41.8	12.5	0.916	7.8
21	58.8	29.7	31.6	24.0	0.940	5.6
23	62.6	44.4	44.2	27.6	1.005	9.0
46	65.1	36.0	43.8	13.0	0.821	8.5
17	66.7	50.4	53.4	26.4	0.944	9.7
37	67.7	35.2	37.8	4.0	0.931	4.5
35	67.9	52.3	60.6	29.4	0.863	9.9
2	87.4	35.8	36.3	21.7	0.986	7.5

* Yield of material finer than 1μ effective diameter, following international method of pretreatment, and pipette method of analysis.

† Yield of material finer than 2μ effective diameter, following international method of pretreatment, and pipette method of analysis.

‡ Yield of material finer than 1μ effective diameter, following shaking over-night with dilute ammonia, and pipette method of analysis.

finer than 1μ equal to less than 80 per cent of the material finer than 2μ, whereas 29 of the soils were found to contain quantities of material finer than 1μ amounting to 90 per cent or more of the material finer than 2μ. The constancy of this ratio between 1 and 2μ material for soils containing different contents of clay is apparent from the values of the fourth and sixth columns of table 1.

DETERMINATIONS OF THE STICKY POINT, THE ROLLING-OUT LIMIT,[4] AND THE NON-STICKY PLASTIC RANGE

The methods used for the determination of the air-dry moisture content and the moisture equivalent have been described in detail elsewhere (4). The sticky point was determined by placing about 10 gm. of air-dry soil on the hand and slowly adding water from a burette until the soil was wet and sticky. The wet soil was then kneaded in the hand by thumb and fingers until, through gradual drying, it reached such a state of moistness that it just failed to stick to the thumb when pressed. The pressure applied by the thumb should be approximately the same at each trial for stickiness. A moisture determination of the mass of kneaded soil was then made immediately by oven drying at 105°C., the moisture content on the dry basis being taken as a measure of the sticky point of the soil. This procedure is practically identical with that described by Keen and Coutts (10, p. 745). Five independent determinations of the sticky point were made by the same worker for each soil. The average range between the highest and the lowest result obtained in each set of five determinations was 1.92 per cent. The lowest such range was 0.5 per cent, and the highest 3.8 per cent. The results given are the averages of the five determinations. Keen and Coutts have pointed out (10, p. 758–760) the differences in results which may be expected between different workers making independent determinations upon the same soils.

The rolling-out limit was measured by using adaptations of Atterberg's (14) original method. For the more clayey soils the procedure was as follows: A rounded mass of about 3 gm. of soil, at approximately the sticky point, was placed on a glazed tile. The mass was then rolled with the flat side of a thin, light piece of wood, by applying a uniform pressure of such magnitude that when the board was moved parallel to the surface of the tile the soil was made to roll between board and tile, meanwhile assuming a cylindrical form which rolled out into a continuous thread. The thread was then destroyed and rolling recommenced. Repetitions of this treatment were continued until, as a result of gradual drying, the cylindrical thread which was formed, broke, upon rolling, into fragments 5 to 15 mm. long by about 2 to 3 mm. in diameter. The moisture content was then determined, and expressed as a measure of the rolling-out limit.

A slightly different method was found necessary for the determination of the rolling-out limit of the more sandy soils, because of the difficulty of rolling such

[4] Determinations of sticky point and rolling-out limit made by M. P. Baltazar.

soils into threads. Ten grams of soil were thoroughly wetted and moulded into a roughly cylindrical form. This cylinder was then placed on a glazed tile and very lightly rolled with the same wooden board as was used for the more clayey soils. During the rolling the surface of the tile was continually wiped dry to hasten the attainment of the end point. Finally, under such treatment, the soil was found to break into loose crumbs upon rolling. This was considered as the rolling-out limit, which was expressed numerically by making a moisture determination of the soil.

TABLE 2

Results of five single-valued measurements for each of 20 soils

SOIL NUMBER	COLLOID BY ADSORPTION	CLAY* 2 MICRONS EFFECTIVE DIAMETER	MOISTURE EQUIVALENT	STICKY POINT	ROLLING-OUT LIMIT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
19	21.4	23.1†	27.3	29.6	27.7
44	21.7	27.6	20.2	20.0	13.5
45	21.7	25.0	18.6	17.2	12.4
43	24.0	23.5	20.1	17.4	15.2
40	27.2	25.5	23.7	24.3	15.5
10	30.8	16.6	26.3	26.0	25.1
25	32.7	29.8	32.2	21.6	13.8
33	33.4	20.0	27.0	24.5	21.7
50	36.8	45.4	27.3	30.1	17.1
5	38.8	32.6	29.1	33.2	22.9
36	40.5	35.0†	29.6	32.5	19.4
16	43.6	36.5	32.6	36.4	25.7
12	45.6	46.3	29.8	30.4	18.7
38	48.2	39.9†	29.2	31.7	18.1
31	49.7	49.4	30.6	33.4	17.9
29	50.0	51.6	34.8	33.9	24.8
3	50.1	37.3	33.7	37.7	29.4
8	50.2	38.3	37.7	36.4	22.6
27	55.0	46.9	28.9	29.1	15.5
21	58.8	31.6	38.6	40.8	30.1

* Following international method of pretreatment.

† Estimated values by means of the regression equation.

Five determinations, made by the same worker for each soil, gave an average range between highest and lowest results of 1.75 per cent for all soils. The lowest range for any set of five soils was 0.4 per cent, the highest 4.2 per cent. The results reported are the average of five separate determinations for each soil.

The non-sticky plastic range was determined by subtracting the rolling-out limit from the sticky point. There seems to be foundation for taking the sticky point as the upper limit of the plastic state, and hence for the measurement of the plastic range. As this may not yet meet with general agreement, and in order to avoid confusion, the term "non-sticky plastic range"

has been used. The word "plastic" strictly is not applied to an adhesive mass of soil, but is applicable for that region lying between the moisture content at which plasticity first becomes apparent (the rolling-out limit) and the

TABLE 3

Results of three single-valued measurements and the ratios existing between some of these, for each of 34 soils

SOIL NUMBER	MOISTURE EQUIVALENT	STICKY POINT	STICKY POINT / MOISTURE EQUIVALENT	ROLLING-OUT LIMIT	ROLLING-OUT LIMIT / MOISTURE EQUIVALENT	NON-STICKY PLASTIC RANGE
	per cent	*per cent*		*per cent*		
65	9.5	13.3	1.41	12.4	1.30	0.9
62	14.8	13.8	0.93	13.4	0.91	0.4
61	15.6	16.3	1.04	11.8	0.76	4.5
66	16.9	14.4	0.86	13.4	0.79	1.0
45	18.6	17.2	0.92	12.4	0.67	4.8
51	18.6	18.5	0.99	19.1	1.03	−0.6
63	19.2	21.3	1.13	17.8	0.61	3.5
43	20.1	17.5	0.87	15.2	0.76	2.3
44	20.2	20.0	0.99	13.5	0.67	6.5
52	21.3	21.8	1.02	15.6	0.73	6.2
54	22.4	26.1	1.18	26.1	1.22	0.0
67	23.1	22.2	0.93	16.6	0.72	5.6
40	23.7	24.3	1.03	15.5	0.65	8.8
53	24.3	24.8	1.02	16.8	0.69	8.0
57	25.4	29.0	1.15	17.8	0.71	11.2
10	26.3	26.0	0.99	25.1	0.95	0.9
33	27.0	24.5	0.91	21.7	0.80	2.8
19	27.3	29.6	1.08	27.7	1.01	1.9
50	27.3	30.1	1.10	17.1	0.63	13.0
27	28.9	29.1	1.01	15.5	0.54	13.6
5	29.1	33.2	1.14	22.9	0.79	10.3
38	29.2	31.7	1.09	18.1	0.62	13.6
36	29.6	32.5	1.10	19.4	0.66	13.1
12	29.8	30.4	1.02	18.7	0.63	11.7
31	30.6	33.4	1.08	17.9	0.58	15.5
25	32.2	21.6	0.67	13.8	0.43	7.8
16	32.6	36.4	1.12	25.7	0.79	10.7
3	33.7	37.7	1.12	29.4	0.87	8.3
29	34.8	33.9	0.97	24.8	0.71	9.1
8	37.7	36.4	0.97	22.6	0.60	13.8
21	38.6	40.8	1.06	30.1	0.78	10.7
64	40.9	42.4	1.04	28.7	0.70	13.7
17	49.3	44.3	0.90	26.7	0.54	17.6
59	50.0	31.2	0.61	16.9	0.34	14.3
Average...	....	....	1.01	....	0.74	....

moistness at which the soil mass becomes adhesive (the sticky point). This range may or may not include the so-called "Fliessgrenze" of Atterberg.

The results of the determinations are contained in tables 2 and 3.

INTERRELATIONS OF THE SINGLE-VALUED EXPRESSIONS

The correlations existing between the colloid by adsorption and the air-dry moisture content, and the content of clay finer than 2μ were examined for 44 soils. In addition the moisture equivalent, sticky point, and rolling-out limit were compared with the colloid by adsorption and with the content of clay finer than 2μ for 20 of the soils, and their coefficients of correlation were determined. Finally, values of the correlation coefficient were obtained between the moisture equivalent and the sticky point, rolling-out limit, and the non-sticky plastic range, respectively, for 34 soils (6, 17, 18).

The results of these calculations are given in table 4, which gives for each correlation, in the last column, the probability that such a correlation would arise by random sampling of an uncorrelated population. The correlations are to be regarded as significant if the probability is low.

In 1916 Alway and Clark (1) suggested the utility of the determination of the hygroscopic moisture of soils as an index of their hygroscopic coefficient, and from numerous careful measurements of both values found that a simple ratio connected the two. They stated that, because of its simplicity, the hygroscopic moisture determination might well be considered as a means of measuring the hygroscopic coefficient wherever the samples were numerous and the labor involved in the direct determination considerable. In connection with their vapor pressure studies in 1925, Puri, Crowther, and Keen (12) pointed out the steepness of the vapor pressure-moisture content curves for soils in the general region of 50 per cent relative humidity, and stated that on that account the air-dry moisture content of a soil might be taken as well as any other single soil constant for characterizing the soil. Since then Keen and Coutts (10) have used the moisture content at 50 per cent relative humidity in that capacity. The high correlation, $r = 0.74 \pm .05$, obtained between measurements of the air-dry moisture content[5] and the colloid by adsorption for 44 soils is in general accord with conclusions to be drawn from the data of Alway and that of the Rothamsted workers.

An approximation to the percentage of colloid by adsorption may be obtained from the air-dry moisture content by means of the regression equation (18),

$$\bar{C} = M_c + r \cdot \frac{\sigma_c}{\sigma_w} (W - M_w)$$

where

W = percentage of air-dry moisture content of particular soil for which an estimated value of colloid content is sought,

[5] No continuous record of the humidity of the air in the laboratory has been obtained. Nearly 500 readings of wet and dry bulb thermometers, kept in the laboratory at some distance from the sample storage room, have since been taken at intervals between 8 a.m. and 5 p.m. for the period September, 1930 to April, 1931. These gave an average relative humidity of 52 per cent.

C = percentage colloid by adsorption,
$\bar{C}$ = estimated value of C,
M_c = mean value of C,
M_w = mean value of W,
σ_c = standard deviation of C,
σ_w = standard deviation of W,
and r = correlation coefficient.

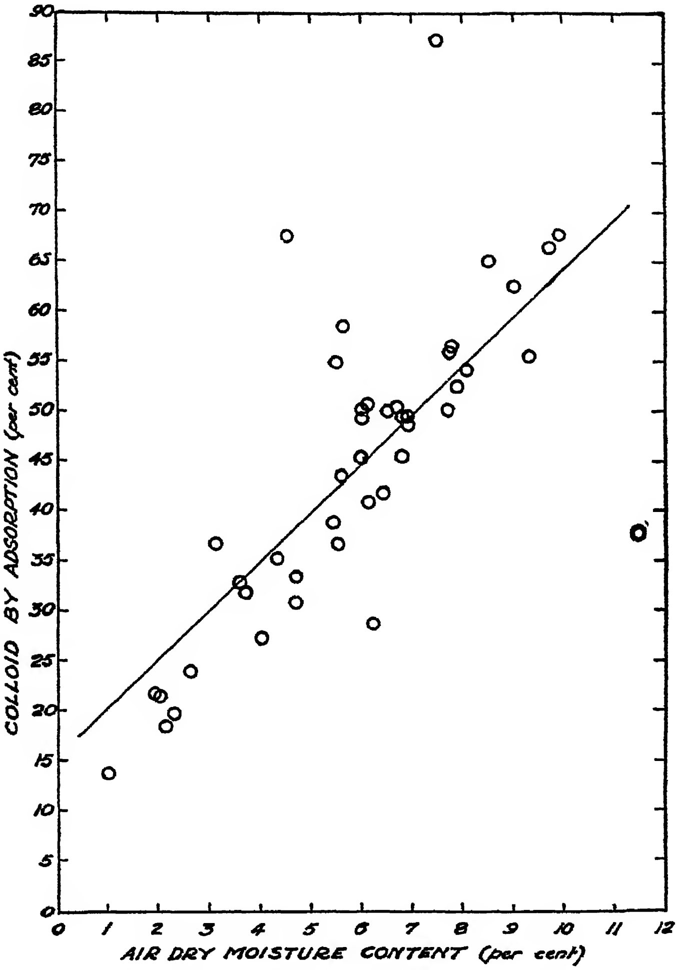


FIG. 1. RELATION BETWEEN AIR-DRY MOISTURE CONTENT AND COLLOID BY ADSORPTION

By calculation from the values of W and C contained in table 1 the regression equation is resolved into the expression, $\bar{C} = 4.91W + 15.6$. The closeness of agreement between estimated and experimental values of the colloid by adsorption is indicated by the scatter diagram and regression line of figure 1.

A somewhat lower degree of correlation is found to exist for these same soils between the colloid content and the content of clay finer than 2μ. This, together with the earlier observations concerning the relative magnitude of the two values, suggests that the measurement of particles having certain settling velocities does not provide a true measure of the colloidally active material.

The high correlations obtained between the colloid by adsorption, and the moisture equivalent and sticky point simply provide further confirmation of the general truth that the higher the colloid content of a soil the higher its water-holding capacity and the higher its moisture content before it becomes

TABLE 4

Correlation coefficients between pairs of single-valued expressions, calculated from data of 1, 2, and 3

VALUES CORRELATED	NUMBER OF SAMPLES USED IN CORRELATION	CORRELATION COEFFICIENT (r)	PROBABILITY* (6)
Colloid by adsorption and clay $<2\mu$	44	0.65 ± 0.06	<0.01
Colloid by adsorption and air-dry moisture content	44	0.74 ± 0.05	<0.01
Colloid by adsorption and moisture equivalent	20	0.83 ± 0.05	<0.01
Colloid by adsorption and sticky point	20	0.83 ± 0.05	<0.01
Colloid by adsorption and rolling-out limit	20	0.42 ± 0.12	$0.05<0.1$
Clay $<2\mu$ and moisture equivalent	20	0.50 ± 0.11	$0.02<0.05$
Clay $<2\mu$ and sticky point	20	0.54 ± 0.11	$0.01<0.02$
Clay $<2\mu$ and rolling-out limit	20	-0.01 ± 0.15	High, no correlation
Moisture equivalent and sticky point	34	0.89 ± 0.02	<0.01
Moisture equivalent and rolling-out limit	34	0.61 ± 0.07	<0.01
Moisture equivalent and non-sticky plastic range	34	0.76 ± 0.05	<0.01

* See page 372.

sticky. The much less close association obtained between the clay finer than 2μ and the moisture equivalent and sticky point indicates that the adsorptive surfaces are of relatively more significance in these particular values than the particle sizes.

From the correlation coefficients of table 4 it appears that the measurement of the colloid by water-vapor-adsorption is a much more significant criterion of the probable physical behavior of a soil than is a measure of its clay finer than 2μ following the hydrogen peroxide-hydrochloric acid pretreatment.

The value of the rolling-out limit is very much less affected by the colloid content and by the clay finer than 2μ.

The values reported in table 3, and their corresponding correlations in table 4. are of much interest. Very close relationships exist between the three single-valued measurements reported, and also between the moisture equivalent and the non-sticky plastic range. These results indicate that a rapid field appraisal may be made of the moisture condition of a given soil in terms of its relative moistness for purposes of decisions concerning moisture supply and timeliness of performing tillage operations. The sticky point represents a higher point on the moisture scale than does the rolling-out limit. Two-thirds of the 34 soils examined had a sticky point within ± 10 per cent of the moisture equivalent. Of the remainder, four soils had sticky points below 90 per cent of the moisture equivalent, ranging from 61 to 86 per cent of that value, and seven had sticky points above 110 per cent of the moisture equivalent, ranging from 112 to 141 per cent of it. The average ratio, sticky point: moisture equivalent, amounted to 1.01. In view of the significance of the moisture equivalent as an index of the moisture holding capacity of the medium textured soils, as shown by Alway and McDole (2), Shaw (15), and others, the rather close agreement with it of the sticky point, a readily determined quantity, is interesting and useful.

The significance of the rolling-out limit is that it represents the lower limit of the plastic consistency for low forces of compression. It is apparently a means of determining the lower moisture limit of easy puddling and of easy reduction of pore space. Over one-half of the 34 soils were found to have a rolling-out limit between 64 and 84 per cent of the moisture equivalent. Nine of the remaining soils had rolling-out limits below 64 per cent of the moisture equivalent and six had values above 84 per cent of it. The average ratio rolling-out limit: moisture equivalent was 0.74.

The close correlation between the non-sticky plastic range and the moisture equivalent indicates that as the texture of the soils becomes finer, as expressed by the moisture equivalent, a wider range is to be expected between the rolling-out limit and the sticky point. That is to say, the lower limit of easy puddling becomes farther and farther removed from the sticky point. By virtue of the relationship existing between the sticky point and the moisture equivalent this also means that for the heavier soils, in terms of absolute moistness, usually the rolling-out limit is farther from the moisture equivalent.

The relationships existing between moisture equivalent, sticky point, and rolling-out limit are shown graphically in figure 2, which is a combined scatter diagram of sticky points and rolling-out limits plotted against moisture equivalents. The non-sticky plastic range is indicated in each case by a vertical bar connecting the sticky point with the rolling-out limit for the same soil. The regression lines for the sticky point and rolling-out limit on the moisture equivalent, with their equations, have been drawn in the figure, assuming linear associations. The shaded portion between the two lines represents the non-

sticky plastic range, and the position which it may be expected to occupy for different values of the moisture equivalent. The divergent nature of the boundaries of this range as the soils increase in fineness of texture is clearly shown. The sticky point and rolling-out limit may be expected to exhibit a tendency to coincide in the vicinity of moisture equivalent = 9.8 per cent. It

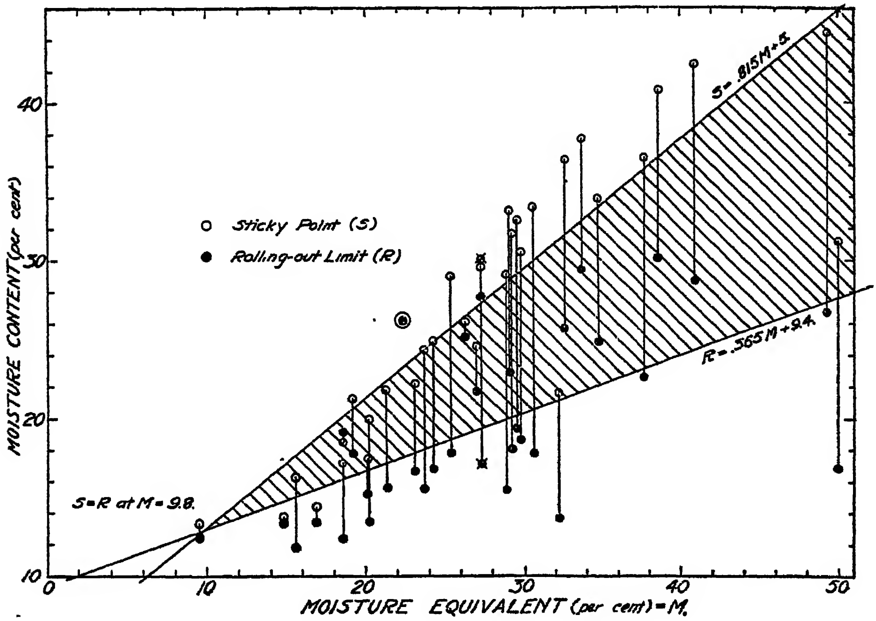


FIG. 2. RELATION BETWEEN MOISTURE EQUIVALENT, STICKY POINT, ROLLING-OUT LIMIT AND NON-STICKY PLASTIC RANGE

is somewhat doubtful whether the inversion of values to the left of $M = 9.8$ per cent would be consistently obtained experimentally, although inversion is known to occur (e.g. soil 51).[6]

SUMMARY

Measurements of several single-valued soil properties; namely, colloid content by adsorption, clay finer than 2μ and clay finer than 1μ following treatment with hydrogen peroxide and hydrochloric acid, clay finer than 1μ following shaking with ammonia water, air-dry moisture content, moisture equivalent, sticky point, and rolling-out limit, were made with a large variety of soils.

[6] Since preparation of this article there has come to our attention a paper by J. C. Russel, "Variations in the B Horizon" in Bul. 9, Rep. 8th Ann. Meeting American Soil Survey Association, pp. 100–112A, March, 1928. In it Russel indicated a relation between clay content and "plasticity number," the latter being the difference between the "Ausrollgrenze" and the "Fliessgrenze." This is in general qualitative agreement with our results.

A comparison of the results for the measurement of colloid by adsorption and clay fractions representing different upper size limits, showed that the adsorption method gave higher results on the whole than were obtained for a measure of the peroxide-hydrochloric acid dispersed clay finer than 2μ, and that the clay finer than 1μ constituted on the average nearly 90 per cent of the total clay having 2μ as the upper limit, for soils of varying clay content. The ammonia pretreatment yielded much lower quantities of clay finer than 1μ than did the peroxide-hydrochloric acid pretreatment. The results indicate that the international method of dispersion and analysis does not permit complete expression of the probable colloid content of a soil, possibly because of indispersible colloid shells at the particle surfaces, and because of the presence of micropores, neither of which is taken into account by a mere expression of size distribution.

Significantly high correlations were found to exist between the moisture equivalent and the sticky point, colloid and moisture equivalent, colloid and sticky point, moisture equivalent and non-sticky plastic range, colloid and air-dry moisture content, colloid and clay finer than 2μ, and moisture equivalent and rolling-out limit, these being here arranged in descending order of their correlation coefficients. Correlations of a lower order were obtained for clay and sticky point, clay and moisture equivalent, and colloid and rolling-out limit. No significant correlation was found between clay and rolling-out limit.

The utility of the relationship between the laboratory air-dry moisture content and the colloid content by adsorption is pointed out.

It is suggested that the sticky point and the rolling-out limit may have considerable significance in the rapid appraisal of soil moisture conditions in the field. A coincidence, or even an inversion, in the values of the sticky point and the rolling-out limit, for which the sticky point is normally the higher, may be expected to occur with soils having amoist ure equivalent in the neighborhood of 9.8 per cent.

REFERENCES

(1) Alway, F. J., and Clark, V. L. 1916 Use of two indirect methods for the determination of the hygroscopic coefficients of soils. *Jour. Agri. Res.* 7: 345–359.

(2) Alway, F. J., and McDole, G. R. 1917 Relation of the water-retaining capacity of a soil to its hygroscopic coefficient. *Jour. Agri. Res.* 9: 27–71.

(3) Anderson, M. S., et al. 1922 Absorption by colloidal and non-colloidal constituents. U. S. Dept. Agr. Bul. 1122.

(4) Bodman, G. B., and Tamachi, M. 1930 Studies of soils in the plastic state. *Soil Sci.* 30: 175–195.

(5) Conclusions of the First Commission meeting at Rothamsted-Harpenden, 1926. Internatl. Soc. Soil Sci. Brno, Czecho-Slovakia, 1927.

(6) Fisher, R. A. 1928 Statistical Methods for Research Workers, ed. 2. London.

(7) Gile, P. L., et al. 1924 Estimation of colloidal material in soils by adsorption. U. S. Dept. Agr. Bul. 1193.

(8) Gile, P. L. 1928 Colloidal soil material. *Soil Sci.* 25: 359–364.

(9) Joseph, A. F. 1927 The determination of soil colloids. *Soil Sci.* 24: 271–274.

(10) Keen, B. A., and Coutts, J. R. H. 1928 "Single value" soil properties: a study of the significance of certain soil constants. *Jour. Agr. Sci.* 18: 740–765.

(11) Olmstead, L. B., Alexander, Lyle T., and Middleton, H. E. 1930 A pipette method of mechanical analysis of soils based on improved dispersion procedure. U. S. Dept. Agr. Tech. Bul. 170.

(12) Puri, A. N., Crowther, E. M., and Keen, B. A. 1925 The relation between the vapour pressure and water content of soils. *Jour. Agr. Sci.* 15: 68–88.

(13) Robinson, W. O. 1922 The absorption of water vapor by soil colloids. *Jour. Phys. Chem.* 26: 647–653.

(14) Russel, J. C., and Wehr, F. M. 1928 The Atterberg consistency constants. *Jour. Amer. Soc. Agron.* 20: 354–372.

(15) Shaw, Charles F. 1927 The normal moisture capacity of soils. *Soil Sci.* 23: 303–317.

(16) Thomas, Moyer D. 1928 Aqueous vapor pressure of soils: IV. Influence of replaceable bases. *Soil Sci.* 25: 485–493.

(17) Tolley, H. R., and Mendum, S. W. 1924 A method of testing farm-management and cost-of-production data for validity of conclusions. U. S. Dept. Agr. Cir. 307.

(18) Wallace, H. A., and Snedecor, G. W. 1925 Correlation and machine calculation. *Iowa State Col. Pub.* 23: (35).

(19) Wiegner, G. 1927 Method of preparation of soil suspensions and degree of dispersion as measured by the Wiegner-Gessner apparatus. *Soil Sci.* 23: 377–390.

PHOTOSENSITIZED OXIDATION OF AMMONIA AND AMMONIUM SALTS AND THE PROBLEM OF NITRIFICATION IN SOILS

G. GOPAL RAO AND N. R. DHAR

University of Allahabad, India

Received for publication August 1, 1930

Most plants get the nitrogen they require from the soil, where it is present in the form of nitrates, ammonium salts, or complex nitrogenous compounds. Ammonium salts and other nitrogenous compounds must first be oxidized to nitrites and nitrates before they can be utilized by the plant. It has long been known that ammonia and ammonium salts are oxidized in the soil to nitrites and nitrates. It is now universally believed that the nitrification in soils is due entirely to the action of bacteria. The biological character of the nitrification process was first indicated by Müller (12) and by Soyka (14). From the painstaking researches of Warington, Frankland, and Winogradsky, we now know that this nitrification is really due to the joint action of two organisms, one of which converts ammonium salts to nitrites and the other of which brings about the oxidation of nitrites to nitrates but has no effect on ammonium salts.

From our experiments on the photosensitized oxidation of ammonia and ammonium salts in the presence of sunlight and various photosensitizers, we are confident that the nitrification in the soil is, at least in part, photochemical in nature, taking place at the surface of various photosensitizers under the influence of sunlight. In the absence of sensitizers there is a noticeable, though very slight, oxidation [compare Dhar and Sanyal (4)]. It has been noted that the same reaction takes place in ultra violet light (3). Under the action of sunlight and in the presence of sensitizers, there is vigorous oxidation of ammonia and ammonium salts to nitrite.

EXPERIMENTAL

Titania, zinc oxide, cadmium oxide, sodium uranate, alumina, and silica were used as photosensitizers. They were prepared by precipitation from the aqueous solutions of the corresponding pure salts and were usually dried, ignited, and finely powdered, for freshly precipitated substances, as a rule, are less active than the ignited materials.

Solutions of ammonia or ammonium salts were exposed to sunlight, with small quantities of the photosensitizer, and a current of air free from nitrous fumes was passed through the solutions, so that the solid photocatalysts were kept in suspension. Glass beakers were generally employed; and as glass transmits only up to 3,500 Å it may be concluded that radiations of shorter

wave length do not take part in our reactions. In all the cases studied, there was no dark reaction.

Nitrite was tested for qualitatively, by the Griess reagent and starch potassium iodide; it was estimated quantitatively, by the usual permanganate method.

Influence of anions

To study the influence of anions, 100 cc. of 0.2 *N* solutions of aqueous ammonia, ammonium carbonate, ammonium phosphate, ammonium chloride, and ammonium sulfate solutions were taken in separate pyrex glass beakers, to each of which was added 1 gm. of Merck's zinc oxide. The solutions were then exposed to the sun for three hours, while a current of air was passed through at an approximately uniform rate. The beakers were kept covered with glass plates. The resulting nitrite was estimated by the permanganate method, which necessitated the use of manganous sulfate whenever chloride was present. The results are shown in table 1.

TABLE 1

Influence of anions on nitrite formation

100 cc. of 0.2 *N* salt solution. Time of exposure to the sun, 3 hours

SALT SOLUTION USED	VOLUME OF 0.025 *N* $KMnO_4$ SOLUTION CORRESPONDING TO THE AMOUNT OF NITRITE FORMED
	cc.
NH_4OH	7.1
$(NH_4)_2CO_3$	3.0
$(NH_4)_2HPO_4$	2.0
$(NH_4)_2SO_4$	2.4
NH_4Cl	2.0

The results shown in table 1 indicate that anions do not have much influence on the rate of oxidation; the pH of the solution, however, seems to have a great influence. The pH values, therefore, of 0.2 *N* aqueous solutions of the salts listed in table 1 were determined.

Salt solution	*pH*
NH_4OH	Above 10
$(NH_4)_2CO_3$	8.1
$(NH_4)_2SO_4$	6.9
$(NH_4)_2HPO_4$	8.0
$(NH_4)Cl$	6.9

On the whole, acidity seems to decrease the rate of oxidation and basicity to increase it; though an exception to this rule is noticed in the case of ammonium phosphate, which requires further investigation.

Comparative experiments in glass and quartz vessels

The rate of oxidation is greater in a quartz vessel than in a glass one. The nitrite formed from 100 cc. of 0.2 *N* aqueous ammonia exposed in a *glass* beaker for three hours corresponds to 7.1 cc. of 0.025 *N* $KMnO_4$, whereas the nitrite formed from the same volume of NH_4OH exposed in a *quartz* beaker for the same length of time, both containing 1 gm. of ZnO, required 11 cc. of the same $KMnO_4$. This fact can be easily explained as follows: Zinc oxide shows marked absorption in the blue, violet, and ultra-violet regions of the spectrum. In sunlight, at least in tropical countries, the ultra-violet region extends to 2,900 Å. When the experiment is carried on in a glass vessel only radiation up to 3,500 Å can be utilized, because glass does not transmit beyond this region, whereas in a quartz vessel all the radiations in the solar spectrum up to 2,900 Å can be utilized. Evidently more of the solar energy is utilized in a quartz vessel than in a glass vessel; and hence the greater rapidity of the reaction.

The accumulation of nitrite seems to have little or no influence on the rate of oxidation of ammonia.

Comparative activities of the various photosensitizers

Semi-quantitative experiments, the results of which are not recorded here, indicate the following order for the photosensitizing activity of the various substances:

$$TiO_2 > ZnO,\ CdO > (Na_2\,U_2O_7) > Al_2O_3 > MgO,\ SiO_2$$

we hope to be able to elucidate the varying activities of these substances from a study of their absorption spectra.

Mechanism of the oxidation

According to Winther (14) oxygen can be ozonized in the presence of zinc oxide by irradiation with light of wave lengths which have no action on oxygen alone. We have found that, when a suspension of zinc oxide in water is exposed to sunlight, ozone and hydrogen peroxide are formed. It is known that ozone interacts with ammonia or ammonium salts according to the equation

$$NH_4OH + O_3 = HNO_2 + 2\ H_2O$$

and the nitrous acid interacts with ammonia giving ammonium nitrite

$$HNO_2 + NH_4OH = (NH_4)NO_2 + H_2O$$

DISCUSSION

These experiments on the photochemical oxidation of ammonia and ammonium compounds to nitrite are remarkably interesting from the point of view of the nitrification in soils. Hitherto it has been believed that the nitrifi-

cation is entirely due to bacteria. We are now convinced that it is, at least in part, photochemical in nature, taking place at the surface of various soil photocatalysts, which absorb the solar radiations. There are some important facts of the nitrification process which do not fit in with the bacterial hypothesis. The present hypothesis not only accounts for these facts, unexplainable by the older hypothesis, but it is also in line with other facts of the nitrification in soils.

The following are some of the important facts unexplainable on the bacterial hypothesis

Omeliansky (13) and Meyerhof (11) have shown that for the bacterial nitrification process a high concentration of ammonia or nitrite is harmful—the optimum concentration is about 0.05 per cent with a second optimum at 0.1 per cent. More than 0.3 per cent interferes with the process. In the so-called niter-spots, a high concentration of nitrite and nitrate, as much as 5 per cent of the bulk of the soil, has been noticed. The biological explanation cannot be entirely sufficient for the accumulation of nitrites and nitrates, for the high concentration of nitrite and nitrate formed should preclude the action of nitrifying organisms in the later stages.

The researches of Traaen (15) McBeth and Smith (10) and Lyon, et al. (8) have shown that the nature of the soil also influences the nitrification.

A periodic variation of the nitrite and nitrate content of soils has been noticed by several workers [Compare Batham, (1)]. The nitrite and nitrate content of soils rises to a maximum in the summer and falls to a minimum in the winter. These results are explained by some as being due to the activating influence of the sun on the nitrifying bacteria. How far this is true cannot be said with certainty at present, but our photochemical hypothesis offers a ready explanation of this phenomenon.

The preceding facts can be explained as follows:

We have shown that the oxidation of ammonium compounds to nitrite takes place at the surface of various oxides, e.g. titania, zinc oxide, cadmium oxide, alumina, and silica in the presence of sunlight. We have also shown that the accumulated nitrite has no influence on the rate of oxidation of ammonium compounds. In the soil, alumina and silica and also titania, undoubtedly occur. The photochemical hypothesis thus sets no limit to the amount of nitrite that can be formed. As such, the accumulation of nitrites and nitrates in large amounts in the nitrite spots is explained.

The influence of soils and the varying activity of different soils also find a ready explanation by this hypothesis. All soils contain alumina and silica; all soils are therefore more or less active in the nitrification process. It has been found that titanium, zinc, and cadmium oxides are very efficient photocatalysts for the nitrification process and are far more active than alumina and silica. Therefore, if the soil contains in addition to alumina and silica, titania or zinc and cadmium oxides or some other efficient photocatalyst as yet undiscovered, it will be very active in the nitrification process and hence very fertile. In consonance with this presumption, it has been recorded by Geilman (5) that most fertile soils contain titania in quantities as large as 0.3 to 0.6 per cent. It also occurs in the ashes of all plants up to 0.27 per cent. According to the same worker, the fertility of the soil depends to some extent on the titanium content of the soil.

The observations made in the present investigation that basicity favors the photochemical oxidation and that acidity is harmful are in agreement with similar observations on the nitrification process in the soil made by various workers [Compare Lyon and Bizzel (8)].

Besides the evidence so far adduced in favour of the photochemical hypothesis, the following also may be mentioned. St. von Bazarewsky (2), Koch (7), and Lyon and his coworkers (9) have found that *the nitrification process is most active near the surface of the soil.* This observation forms a necessary deduction from our photochemical hypothesis, for most the of light falling is absorbed by the outermost layers of the soil and little is allowed to pass through. As light is essential for the photochemical nitrification, it is but natural that there is the greatest oxidation where there is the maximum absorption of radiant energy.

SUMMARY

Oxidation of ammonia and its compounds in solution to nitrite has been effected by sunlight in the presence of photosensitizers. The velocity of oxidation is greater in quartz vessels than in glass vessels.

The various photosensitizers fall into the following order with respect to their activity:

$$TiO_2 > ZnO, CdO > Na_2U_2O_7 > Al_2O_3 > SiO_2$$

Basicity favors the oxidation, and acidity decreases the velocity of oxidation.

On the basis of the foregoing experiments, a new mechanism for the nitrification in soils has been suggested. We believe that the nitrification in soils is, at least in part, photochemical in nature, taking place at the surface of various photocatalysts present in the soil under the influence of sunlight.

The bacterial explanation of the soil nitrification process has been shown to be inadequate. Facts which do not fit in with this hypothesis, have been shown to be explicable on the photochemical hypothesis. We believe that the bacterial and photochemical processes take place side by side.

Further work is in progress in this line.

REFERENCES

(1) Batham, H. N. 1930 Periodicity of the nitrate content of soils. *Proc. Indian Sci. Cong.* 17: 35.

(2) Bazarewsky, St. von 1908 Nitrification and denitrification in soils. Rev. in *Neues Jahrb. Min. Geol. u. Paläontol* 2: (2) 186.

(3) Berthelot, D., and Gaudechon, H. 1911 Physico-Chimie. La nitrification par les rayons ultraviolets. *Compt. Rend Acad. Sci.* (*Paris*) 152: 522–524.

(4) Dhar, N. R., and Sanyal, R. P. 1925 Photosynthesis in tropical sunlight. *Jour. Phys. Chem.* 29: 926–934.

(5) Geilmann, 1920 Distribution of titanium in soils and plants. *Jour. Landw.* 68: 107–124.

(6) Kelley, W. P. 1916 Nitrification in semiarid soils. *Jour. Agr. Res.* 7: 417–437.

(7) Koch, A. 1911 Formation of nitrates in cultivated soil. *Jour. Landw.* 59: 293–315.

(8) Lyon, T. L., and Bizzell, J. A. 1909 Some conditions favoring nitrification in soils. *Science* (n.s.) 30: 773–774.

(9) Lyon, T. L., Bizzell, J. A., and Conn, H. J. 1913 An examination of some more productive and some less productive sections of a field. N. Y. (Cornell) Agr. Exp. Sta. Bul. 338.

(10) McBeth, L. G., and Smith, N. R. 1914 The influence of irrigation and crop production on soil nitrification. *Centbl. Bakt.* (II) 40: 24–51.

(11) Meyerhof, O. 1917 Respiratory process of nitrifying bacteria. The respiration of the nitrite organisms and the influence of chemical substances. *Pflüger's Archiv.* 166: 240.

(12) Müller, A. 1877 *Landw. Vers. Stat.* 6: 263. (Reference from Mellor's comprehensive treatise on inorganic and theoretical chemistry, vol. 8, p. 357.)

(13) Omeliansky, W. 1899 Ueber die Nitrifikation des organischen Stickestoffes. *Centbl Bakt.* (II) 5: 473–490.

(14) Soyka, J. 1877 *Zeit. Biol.* 14: 449. (Reference from Mellor's comprehensive treatise on inorganic and theoretical chemistry, vol. 8, p. 357.)

(15) Traaen, A. E. 1916 Über den Einfluss der Feuchtigkeit auf die Stickstoffunsetzungen im Boden. *Centbl. Bakt* (II) 45: 119–135.

(16) Winther, C. 1922 The formation of ozone by optical sensitization. *Ztschr. Wiss. Photochem.* 21: 168–174.

CHANGES PRODUCED IN NITROGENOUS COMPOUNDS BY RHIZOBIUM MELILOTI AND RHIZOBIUM JAPONICUM[1]

GEORGE GORDON POHLMAN

Iowa State College of Agriculture and Mechanic Arts

Received for publication July 19, 1930

During the past few years there have been many investigations of the physiological activities of the legume bacteria in the attempt to gain a better understanding of their species relationships and nitrogen fixing activities. Although much has been learned of the physiology of these organisms there are still many phases of their activity which need further study.

Changes in the nitrogenous compounds in media in which the legume bacteria have been grown have been noted by certain investigators. Hills (5) and Allison (1) noted the disappearance of nitrates in solutions inoculated with certain species of Rhizobium. Lewis and Nicholson (7) found a utilization of nitrates, accompanied by the production of nitrites by certain strains of *Rhizobium meliloti* and *Rhizobium japonicum*. The production of nitrites was also noted by Leonard (6).

Many investigators found a more vigorous growth in media containing combined nitrogen, but did not determine to what extent the utilization of nitrogen was responsible for the increased growth. Müller and Stapp (8) found that potassium nitrate, sodium nitrate, ammonium chloride, ammonium sulfate, ammonium nitrate, asparagine, aspartic acid, and uric acid served as suitable sources of nitrogen for the legume bacteria. Urea was found to be somewhat suitable as a nitrogen source, alanine and leucine were suitable for certain groups, and glycocoll and hippuric acid did not serve as suitable nitrogen sources for the legume bacteria. These authors also noted that the legume bacteria produced a proteolytic enzyme having a weak action. The presence of an oxidizing enzyme having a weak action was noted by Fred (4) but he was unable to demonstrate either invertase or proteolytic reducing enzymes.

The recent work of Baldwin, Fred, and Hastings (2) suggested that there may be a relationship between the various species of the legume bacteria and the proteins present in the seeds of leguminous plants. It seems then, that a study

[1] Part of a thesis submitted in partial fulfillment of the requirements of the degree of doctor of philosophy at the Iowa State College.

The writer takes this opportunity to thank Dr. P. E. Brown for his helpful suggestions in planning the experimental work and in the preparation of the manuscript, and Dr. R. H. Walker for the help which he has given during the progress of the work.

of the changes brought about by the legume bacteria in media containing nitrogen may have some significance. The results of investigation along this line are reported here.

EXPERIMENTAL

In the first experiments an attempt was made to determine whether there was a difference in the nitrogen changes produced by *Rhizobium meliloti* and *Rhizobium japonicum*. Since no nitrogen was fixed in earlier experiments, compounds containing nitrogen were added in all of these tests. The basic medium consisted of

Mannitol	10.0 gm.
K_2HPO_4	0.5 gm.
$MgSO_4$	0.2 gm.
NaCl	0.2 gm.
$CaCO_3$	3.0 gm.
$FeCl_3$	Trace
$MnSO_4$	Trace
Distilled water	1,000 cc.

The compounds tested were glycocoll, asparagine, tyrosine, peptone, and urea. With the exception of peptone and urea these were added at the rate of 0.5 gm. to a liter of solution. Urea was added at the rate of 2 gm. a liter and peptone at the rate of 1 gm. a liter. After the reaction of the media had been adjusted to pH 7.0, 100-cc. portions were placed in 500-cc. Erlenmeyer flasks, sterilized, and duplicate flasks inoculated as follows —

107. *Rhizobium meliloti*
112. *Rhizobium meliloti*
406. *Rhizobium japonicum*
407. *Rhizobium japonicum*

These organisms had previously been tested for ability to produce nodules on the host plant and for purity in milk and on potato slants. After three weeks incubation at room temperature the solutions were analyzed for total nitrogen, ammonia, nitrates, nitrites, and amino acid nitrogen. The methods used were as follows:

Total nitrogen. The determination of total nitrogen was made on a 25-cc. aliquot from each of the solutions. A modified Kjeldahl method was used in which a salt mixture containing sodium sulfate, copper sulfate, and ferrous sulfate was added to hasten digestion. In the solutions which contained nitrates or nitrites in measurable amounts the method of Davisson and Parsons (3) was used.

Ammonia. An aeration method was used for the determination of ammonia. Twenty-five cubic centimeters of the sample was placed in a large test tube, 0.5 gm. of sodium carbonate added, and the solution aerated. The ammonia was collected in 0.1 *N* sulfuric acid and titrated.

Nitrites. The test for nitrites was made according to the sulfanilic acid-alpha-naphthylamine method.

Nitrates. Nitrates were determined on the same sample used for the nitrite determination. A small amount of zinc dust was added to reduce the nitrates to nitrites, which could then be detected by the aforementioned reagents.

Amino acids. The Sorenson formol titration method was used for the determination of amino acids. For this determination, 25 cc. of the sample was taken. The amino acid utilized was obtained by subtracting the sum of the ammonia produced and the amino acid remaining from the amino nitrogen present in the check solutions.

These determinations were carried out as rapidly as possible in order to prevent any change due to contamination after the solutions had been exposed to the laboratory air.

The results obtained in the study of the changes produced in glycocoll appear in table 1. Both strains of *Rhizobium meliloti* utilized this amino acid. Part of the loss of amino acid nitrogen can be accounted for by the ammonia produced, but some of it was apparently utilized by these organisms in the

TABLE 1

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 0.5 gm. of glycocoll to a liter

(Results expressed in milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	0.28	9.24	None	8.68
Check	0.56	8.96	None	8.96
107	4.48	3.36	1.68	9.52
107	4.20	2.80	2.52	9.52
112	6.16	0.56	2.80	9.80
112	6.16	0.84	2.52	8.96
406	0.56	8.68	0.28	9.80
406	0.28	9.24	−0.14	9.52
407	0.28	9.24	−0.14	8.96
407	0.28	9.24	−0.14	9.52

building up of their protein. The solutions inoculated with the two strains of *Rhizobium japonicum* did not show any evidence of growth during the period. Apparently the glycocoll was toxic to them in this concentration.

The variations in total nitrogen were within the limit of experimental error. They do show, however, that no appreciable amounts of nitrogen were gained or lost from the solutions during the period of incubation. Qualitative tests showed only traces of nitrites in the check solutions and in the solutions inoculated with organisms 112, 406, and 407. The small amount of nitrite present had apparently been utilized by organism 107. No nitrates were found in any of the solutions.

The changes in urea are shown in table 2. Some ammonia was produced by all of the organisms tested, but there was no significant difference between the two species. The loss of nitrogen found in some of the solutions was probably due to a loss of ammonia, as all of the organisms tested made this medium more alkaline. Nitrites and nitrates were absent from all of the cultures. No amino acid nitrogen could be detected in any of the solutions.

Table 3 shows the changes produced in asparagine. The production of ammonia and the decrease in amino acid nitrogen were much greater by the one strain of soybean bacteria than by the alfalfa bacteria. The Sorenson formol titration method was apparently not well suited for the determination of amino acid nitrogen in asparagine, but the results were at least comparative. In asparagine one-half of the nitrogen is present in an amino group and the other half in an amido group. The determination did not show which of these groups was changed, but apparently both were acted upon by *Rhizobium japonicum* since the amount of nitrogen changed to ammonia was greater than the amount of nitrogen present as either amino or amido nitrogen. It is probable

TABLE 2

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 2.0 gm. of urea to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	TOTAL NITROGEN
Check	6.16	None	95.8
Check	5.88	None	95.5
107	9.80	None	89.1
107	9.80	None	88.5
112	17.91	None	96.4
112	17.63	None	94.0
406	9.80	None	90.5
406	9.80	None	89.8
407	11.20	None	90.5
407	10.92	None	90.5

TABLE 3

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 0.5 gm. of asparagine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID CHANGED*	TOTAL NITROGEN
Check	Trace	4.76	0	11.20
Check	Trace	4.76	0	10.58
107	2.52	3.08	1.68	11.06
107	3.08	2.52	2.24	10.92
112	2.52	3.08	1.68	11.34
112†	....	....	....	.....
406†	....	....	....	.....
406†	....	....	....	.....
407	6.16	0.00	4.76	10.36
407	6.16	0.00	4.76	10.36

* Represents difference between total amino acid in check and inoculated solutions.

† Culture contaminated.

also that the two strains of *Rhizobium meliloti* changed some of the nitrogen present in both groups. There was no significant change in the total nitrogen content of any of the solutions, and no nitrates or nitrites were produced in this medium.

The changes produced in tyrosine are shown in table 4. Although there appeared to be a slight utilization of this amino acid there was no apparent difference in the ability of the two species to utilize tyrosine. No ammonia, nitrates or nitrites could be detected in any of the solutions. An interesting difference in the color of the solutions was noted. The check solutions were clear and colorless, those inoculated with the organisms 107 and 112 were golden brown, and those inoculated with 406 and 407 were a very dark brown. An explanation of the differences in colors produced is given later.

TABLE 4

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 0.5 gm. of l-tyrosine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	None	2.80	None	Not determined
Check	None	2.80	None	
107	None	2.22	0.58	
107	None	1.68	1.12	
112	None	1.96	0.84	
112	None	1.96	0.84	
406	None	1.96	0.84	
406	None	1.96	0.84	
407	None	1.96	0.84	
407	None	1.96	0.84	

TABLE 5

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of peptone to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	None	0.84	None	7.28
Check	None	0.84	None	7.28
107	None	0.42	0.42	7.84
107	None	0.56	0.28	7.84
112	None	0.28	0.56	7.28
112*	......	....	....	....
406	None	Lost	Lost	7.84
406	None	0.56	0.28	7.84
407	None	0.56	0.28	7.56
407	None	0.56	0.28	7.84

* Culture contaminated.

Table 5 shows the nitrogen changes which were brought about in a peptone solution. A small amount of amino acid nitrogen was measured by the formol titration method, part of which was utilized by the organisms tested. No ammonia, nitrates, or nitrites were produced in the medium. There was no appreciable increase or decrease in the total nitrogen content of the solutions.

In view of the differences found in the changes in the nitrogenous compounds tested in the foregoing experiments it was decided to carry out further tests, using different strains of the same two species of bacteria, and including a study of still other nitrogenous compounds. The organisms used were the following:

113. *Rhizobium meliloti*	
130. *Rhizobium meliloti*	Type A strain
131. *Rhizobium meliloti*	Type B strain
132. *Rhizobium meliloti*	Type B strain
133. *Rhizobium meliloti*	
413. *Rhizobium japonicum*	Type A strain
414. *Rhizobium japonicum*	Type B strain
415. *Rhizobium japonicum*	Type B strain
416. *Rhizobium japonicum*	Type A strain

The four strains of *Rhizobium meliloti* (strains 130, 131, 132, and 133) and the four strains of *Rhizobium japonicum* (strains 413, 414, 415, and 416)[2] had previously been divided into two types as shown by Stevens (9) and Wright (11). The type A strains were rapid growers and fixed larger amounts of nitrogen in the host plant than the type B strains. Organism 113 was chosen because it produced a very marked increase in acidity when grown in yeast mannitol solution. Organism 133, although not classified by Stevens, appeared to be more like the type A strain 130 than the type B strains.

The organisms were tested for ability to inoculate the host plant and for purity on milk.

The nitrogenous compounds used in these tests included some aliphatic amino acids, some aromatic amino acids; some compounds having an amido group, such as asparagine and urea; and two inorganic nitrogenous compounds, ammonium sulfate and potassium nitrate.

The basic medium used was the same as in the previous tests, but the amount of the nitrogenous compound added was increased to 1.0 gm. to each liter. The incubation period was increased to five weeks. At the end of the incubation period yeast mannitol agar slants were inoculated with a suspension from each flask, and the type of growth was noted to verify the purity of the cultures at the end of the experiment. The species of Rhizobium tested have a characteristic type of growth on yeast mannitol agar slants which helps to differentiate them from other organisms.

The methods of analyses were the same as were used in the previous tests, except that the micro Van Slyke method (10) was used for the determination of

[2] Obtained through the courtesy of Dr. I. L. Baldwin of the Wisconsin Agricultural Experiment Station.

amino acid nitrogen. This was slightly modified by substituting the large deaminizing bulb from the macro apparatus, thus permitting the use of a 10-cc. aliquot instead of the 2-cc. aliquot used in the micro method. By this change more accurate determinations were made possible. The determination of nitrates and nitrites in the solutions receiving additions of potassium nitrate was made by reducing the nitrates and nitrites to ammonia by means of Devarda's alloy and distilling the ammonia produced into standard acid.

The changes produced in the glycocoll solution by the organisms tested are shown in table 6. The concentration of glycocoll used was apparently above

TABLE 6

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of glycocoll to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	None	21.18	None	20.9
Check	None	21.10	None	23.3
113	None	21.80	−0.66	22.1
113*	......	.....	......	....
130	None	22.00	−0.86	22.1
130*	......	.....	......	....
131	None	22.10	−0.96	22.4
131	None	22.00	−0.86	22.1
132	None	20.86	0.28	21.5
132*	......	.....	....	....
133	None	21.60	−0.46	23.5
133*	......	.....	....	....
413	None	21.08	0.06	23.0
413	None	22.05	−0.91	23.8
414	None	20.98	0.16	22.4
414	None	21.40	−0.26	22.7
415	None	21.62	−0.48	22.4
415*	......	.....	....	....
416	None	21.30	−0.16	22.1
416	None	21.08	0.06	21.5

* Culture contaminated.

the toxic limit of all of the organisms tested, as the solutions did not become turbid, and no growth was apparent. There were no changes in the nitrogen compounds present.

The nitrogen changes in a solution containing dl-alanine are shown in table 7. There was a much higher production of ammonia and a higher utilization of amino acid nitrogen in the solutions inoculated with the type A strain 130 and with organism 133 than in the solutions containing the type B organisms. Organism 113 was intermediate in action.

There was no change in the nitrogen present in the solutions inoculated with

the type A strains of *Rhizobium japonicum*, the organisms apparently being destroyed by the high concentration of alanine. The type B strains, however, apparently made some growth and utilized some of the amino acid nitrogen. Only a small amount of ammonia was produced by the type B strains. Nitrates and nitrites were absent from all of the solutions. The slight variation in the total nitrogen content of the solutions was probably within the limit of experimental error.

TABLE 7

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-alanine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	0.56	16.38	None	17.42
Check	0.56	16.58	None	17.42
113	1.96	13.26	1.82	16.80
113	2.80	13.12	1.12	17.12
130	3.92	9.28	3.84	16.96
130	4.76	8.44	3.84	17.42
131	1.12	14.84	1.08	17.26
131	1.12	15.28	0.64	18.20
132	0.56	16.40	0.08	17.92
132	0.56	16.96	−0.48	16.80
133	3.92	9.60	3.52	17.12
133	2.80	10.94	3.30	17.92
413	0.28	17.10	−0.62	16.80
413	0.28	16.92	−0.44	17.42
414	0.84	13.34	2.86	16.68
414	0.84	14.28	1.92	16.80
415	0.28	15.06	1.42	16.80
415	1.40	13.82	1.82	17.92
416	0.28	16.36	0.12	17.63
416	0.28	16.88	−0.40	18.20

Table 8 shows the nitrogen changes produced in dl-alpha-amino-n-butyric acid. The production of ammonia and the utilization of the amino acid nitrogen were again much greater in the solutions inoculated with the alfalfa organisms 130 and 133. There was a very small amount of ammonia produced by the type B alfalfa organisms and a slightly larger amino acid utilization. The soybean bacteria were apparently not able to utilize this amino acid although one result does seem to show a slight utilization. The cultures were all free from nitrates. Traces of nitrites were present in the check solutions but no nitrites could be detected in any of the inoculated solutions. The variations in the total nitrogen content were too small to be of significance.

The changes in dl-valine are shown in table 9. The results obtained with both species of Rhizobium were variable and did not show any appreciable util-

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-amino-n-butyric acid to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	None	14.68	None	14.85
Check	None	14.68	None	15.12
113	0.56	6.76	7.36	15.40
113	0.56	13.58	0.54	15.12
130	1.12	11.48	2.08	15.68
130*	....	.....	....	.....
131	0.56	13.21	0.91	15.68
131*	....	.....	....	.....
132	0.28	13.20	1.20	14.57
132	0.28	13.25	1.15	14.85
133	2.80	8.23	3.65	15.12
133	3.08	7.79	3.81	16.53
413	None	14.54	0.14	15.68
413*	......	.....	....	.....
414	None	14.65	0.03	14.57
414	0.28	13.67	0.73	15.12
415	None	14.80	0.12	15.96
415*	......	.....	....	.....
416	None	14.65	0.03	14.28
416*	......	.....	....	.....

* Culture contaminated.

TABLE 9

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-valine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	0.28	13.31	None	12.60
Check	0.28	13.42	None	13.16
113	0.28	13.57	−0.21	12.05
113*	....	.....	....	.....
130	0.56	12.82	0.26	12.60
130	0.56	12.86	0.22	13.16
131	0.28	13.57	−0.21	13.44
131*	....	.....	....	.....
132	0.28	13.00	0.36	13.72
132	0.28	12.96	0.40	13.44
133	0.56	12.99	0.09	14.56
133	0.28	13.17	0.19	Lost
413	0.28	13.57	−0.21	14.56
413*	....	.....	....	.....
414	0.28	13.57	−0.21	13.72
414	0.28	13.68	−0.32	13.16
415	0.28	13.31	0.05	13.44
415	0.28	13.21	0.15	14.56
416	0.28	13.14	0.22	14.28
416*	....	.....	.....	.....

* Culture contaminated.

ization of amino acid nitrogen nor any production of ammonia. The slight increase in total nitrogen in many of the inoculated solutions was not significant. There were no nitrates nor nitrites present in any of the solutions.

The nitrogen changes brought about in a solution containing d-glutamic acid are shown in table 10. Although there was no ammonia present in any of the cultures after the 5-week incubation period there was an appreciable utilization of the amino acid nitrogen in all of the tests. This was small in the solutions inoculated with *Rhizobium meliloti,* and there was no difference between the various strains of organisms in this species. The utilization was slightly

TABLE 10

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of d-glutamic acid to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	None	10.42	None	10.08
Check	None	10.14	None	10.08
113	None	9.44	0.84	11.48
113	None	9.82	0.46	10.08
130	None	9.71	0.57	10.63
130*	......	....	....	.....
131	None	9.46	0.82	10.92
131	None	9.36	0.92	10.08
132	None	9.19	1.09	10.63
132	None	9.19	1.09	10.15
133	None	9.41	0.87	11.54
133	None	9.25	1.03	10.50
413	None	8.77	1.51	10.08
413	None	8.56	1.72	10.63
414	None	0.26	10.02	10.63
414	None	Lost	Lost	10.36
415	None	0.53	9.75	12.88
415	None	0.69	9.59	10.92
416	None	8.82	1.46	9.52
416*	......	....	....	.....

* Culture contaminated.

greater by the type A strains of *Rhizobium japonicum,* and very much greater by organisms 414 and 415, which are type B strains. These latter had utilized nearly all of the amino acid nitrogen. Qualitative tests showed small amounts of nitrites in the check solutions, but none in any of the inoculated solutions, indicating a utilization of the small amount present. Nitrates were not found in any of the solutions. The total nitrogen determinations showed no appreciable gain or loss in the nitrogen content during the incubation period.

Table 11 shows the nitrogen changes produced in l-cystine solution. In order to provide a solution of cystine having the desired concentration it was

necessary to add hydrochloric acid to the medium, thus producing a salt which was readily soluble. Sufficient sodium hydroxide was then added to bring the reaction up to a pH of 7.0. There was apparently a small amount of ammonia in the reagents which was utilized by most of the organisms. Although the Van Slyke determination did not measure all of the amino acid nitrogen it was apparent that some amino nitrogen was utilized by all of the cultures tested, the amounts varying more widely within the species than between the two species tested. Of the solutions inoculated with *Rhizobium meliloti* the one in which culture 130 (the type A strain) was growing showed a greater utilization

TABLE 11

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of l-cystine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	0.56	6.88	None	9.52
Check	0.84	6.54	None	11.21
113	None	6.06	0.65	10.37
113	None	5.94	0.77	10.37
130	None	3.64	3.07	12.32
130*	......	....	....	.....
131	None	5.77	0.94	10.64
131	0.56	5.27	1.44	9.52
132	None	5.93	0.78	11.77
132	None	6.04	0.67	11.21
133	None	6.12	0.59	11.21
133	None	6.12	0.59	11.77
413	None	5.37	1.34	11.64
413	None	5.26	1.45	8.41
414	0.56	3.62	3.09	11.49
414	0.28	3.86	2.85	10.09
415	None	4.57	2.14	8.69
415	None	4.67	2.04	9.25
416	None	5.16	1.55	Lost
416	None	5.74	0.97	10.09

* Culture contaminated.

of amino acid nitrogen than those containing the type B strains. Organisms 113 and 133 utilized about the same amount of amino nitrogen as the type B strains.

In the solutions inoculated with *Rhizobium japonicum* the reverse was true, the type B strains utilizing more than the type A strains, but the differences between these two were not large. The differences in the total nitrogen content were of little significance. Nitrates were not present in any of the solutions. Traces of nitrites were found only in the check solutions, the small amount present evidently being utilized in the inoculated solutions.

The nitrogen changes in l-tyrosine solution are given in table 12. There was no ammonia present in any of the solutions at the end of the 5-week incubation period. The amino nitrogen utilized varied from 0.60 mgm. in culture 113 to 2.73 mgm. in culture 416. The color of the solutions at the end of the incubation period were found to be much the same as in the previous test with tyrosine, except that the two strains of soybean bacteria 414 and 415 did not cause any change in the color of the medium. The amount of growth seemed to be much greater in the solutions inoculated with 414 and 415 than in any of the others, but the utilization of amino acid nitrogen was less than in the other strains of soybean organisms, and only slightly higher than in the solutions inoculated with the type A alfalfa organisms. Apparently the production of color was not dependent upon the utilization of amino acid nitrogen, but rather was the result of enzyme action. There were no nitrates present in any of the solutions, and the differences in the total nitrogen content, although usually showing a slight loss in nitrogen present, were not of any great importance. Nitrites were present in small amounts in the check solutions but had entirely disappeared from the inoculated media.

TABLE 12

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of l-tyrosine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	None	8.17	None	9.10
Check	None	8.69	None	10.08
113	None	7.83	0.60	7.74
113	None	7.40	1.03	8.68
130	None	7.20	1.23	8.40
130	None	7.42	1.01	8.96
131	None	6.22	2.21	8.96
131	None	6.01	2.42	8.68
132	None	6.05	2.38	9.52
132	None	5.84	2.59	10.08
133	None	6.86	1.57	8.40
133	None	7.10	1.33	8.96
413	None	5.82	2.61	8.68
413	None	5.88	2.55	7.74
414	None	6.31	2.12	7.74
414	None	6.58	1.85	7.56
415	None	6.90	1.53	7.56
415	None	6.94	1.49	8.96
416	None	5.79	2.64	7.28
416	None	5.70	2.73	7.28

The changes produced in dl-phenylalanine are shown in table 13. The amounts of amino acid nitrogen utilized varied from —0.03 mgm. to 1.06 mgm.

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-phenylalanine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	AMINO ACID UTILIZED	TOTAL NITROGEN
Check	None	9.25	None	9.96
Check	None	9.30	None	9.96
113	None	8.92	0.35	9.96
113	None	9.25	0.02	9.80
130	None	8.98	0.29	10.90
130	None	8.98	0.29	9.96
131	None	9.14	0.13	10.27
131	None	9.30	−0.03	10.58
132	None	9.25	0.02	9.34
132*	......	....	......	....
133	None	8.21	1.06	9.80
133	None	8.85	0.42	9.50
413	None	9.28	−0.01	9.65
413	None	8.64	0.63	9.96
414	None	8.48	0.79	9.25
414*	......	....	......	....
415	None	9.30	−0.03	9.65
415	None	9.20	0.07	10.27
416	None	9.02	0.25	9.25
416	None	8.87	0.40	10.12

* Culture contaminated.

TABLE 14

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of p-amino benzoic acid to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	TOTAL NITROGEN
Check	None	Not determined	10.36
Check	None		10.08
113	None		10.08
113	None		10.36
130	None		10.92
130	None		10.08
131	None		9.80
131	None		9.24
132	None		10.36
132	None		10.08
133	None		10.64
133	None		9.52
413	None		10.64
413	None		9.80
414	None		9.52
414	None		9.52
415	None		8.96
415	None		9.52
416	None		9.52
416	None		9.80

There did not seem to be any consistant relationship between the organism and the amount of amino acid nitrogen utilized. No ammonia, nitrates, or nitrites were found in any of the solutions. The variations in the total nitrogen content were within the experimental error.

The results obtained with p-amino benzoic acid are shown in table 14. Neither *Rhizobium meliloti* nor *Rhizobium japonicum* was able to produce any ammonia from this amino acid. The determination of the amount of amino nitrogen could not be made by the Van Slyke method as the amino group in positions other than on the alpha carbon atom reacts very slowly. No nitrates

TABLE 15

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of asparagine to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	TOTAL NITROGEN
Check	0.56	23.50
Check	0.56	23.50
113	3.92	24.06
113	3.92	23.24
130	4.48	23.50
130	4.20	24.06
131	2.80	23.80
131	4.76	23.50
132	4.48	24.06
132	4.48	23.24
133	3.36	23.80
133	2.80	23.80
413	1.96	24.50
413	2.52	22.96
414	10.92	18.75
414	10.36	19.22
415	10.36	18.48
415	5.60	17.92
416	3.36	24.64
416	2.80	24.06

nor nitrites were present. The variations in the total nitrogen content of the solutions, although seeming to indicate the loss of small amounts of nitrogen from the inoculated solutions, were small. The four solutions containing the type B strains of soybean bacteria (414 and 415) showed a dark brown to black color at the end of the incubation period. This was even darker than the color produced in tyrosine by the type A strains of *Rhizobium japonicum.*

Table 15 shows the changes in asparagine caused by *Rhizobium meliloti* and *Rhizobium japonicum.* There was a marked production of ammonia by all of the organisms tested, the greatest production occurring in the solutions inoculated with the type B strains of soybean organisms. The results of the amino

acid determinations were not included in the tables because the tests were made before the removal of the ammonia, and include a part of it. They did show in all cases a slight increase in the amount determined as a result of inoculation, indicating that some of the ammonia measured must have been produced from the amido group. There was little difference in the production of ammonia by the alfalfa bacteria tested or by the type A strains of soybean bacteria. However, the amount of ammonia produced by the type B strains of *Rhizobium japonicum* was much greater than by any of the other organisms tested. The loss of ammonia probably was the cause of the decrease in the total nitrogen

TABLE 16

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of urea to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMINO ACID TOTAL	TOTAL NITROGEN
Check	4.20		51.8
Check	3.92		50.9
113	16.23		22.6
113	16.80		21.0
130	17.08		24.6
130	18.20		22.1
131	13.17		21.0
131	15.12		26 8
132	7.78		22.7
132*	....	Not determined	....
133	6.44		21.8
133	6.44		30 0
413	6.16		42.0
413	5.88		42.5
414	4.76		41.7
414	4.76		41.7
415	5.03		35.8
415	Lost		35.2
416	4.76		38.4
416	4.48		40 3

* Culture contaminated.

content in the solutions inoculated with cultures 414 and 415. Nitrates and nitrites were absent from all of the solutions.

The nitrogen changes in a solution containing urea are shown in table 16. The production of ammonia, although significant in all of the tests, was much greater in the solutions inoculated with strains 113, 130, and 131 of *Rhizobium meliloti*. Amino nitrogen was not determined. There was a significant loss of nitrogen in all of the inoculated solutions, probably due to a loss of ammonia. This loss was much greater in the solutions inoculated with the alfalfa bacteria.

Table 17 shows the changes produced in a solution containing ammonium sulfate. A small amount of ammonia was utilized by all of the organisms, the amount varying considerably. Usually the amount utilized was small, often less than the amount of nitrogen utilized by the same organisms grown in amino acid solutions. There was no significant difference in the utilization by the two species nor by the different types within the species. The determination of total nitrogen did not show any marked change in the nitrogen content of the inoculated solutions. Nitrates and nitrites were not found in any of the solutions.

TABLE 17

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of ammonium sulfate to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA	AMMONIA UTILIZED	TOTAL NITROGEN
Check	21.20	None	22.02
Check	21.20	None	21.32
113	20.17	1.03	21.82
113	5.60	15.60	21.26
130	19.22	1.98	22.65
130	19.60	1.60	22.65
131	17.36	3.84	23.40
131	14.28	6.92	22.65
132	19.22	1.98	21.56
132	19.22	1.98	21.82
133	19.22	1.98	21.82
133*	.....	....	.....
413	19.88	1.32	21.26
413*	.....	....	.....
414	16.80	4.40	20.22
414	17.36	3.84	20.22
415	18.20	3.00	22.40
415	18.20	3.00	21.26
416	15.68	5.52	21.00
416	12.60	8.60	21.26

* Culture contaminated.

The changes in the form of nitrogen in solutions containing potassium nitrate are shown in table 18. The amounts of amino acid and ammonia were small in all of the solutions and it is probable that all of the nitrogen was present in the amino form, which was produced during the utilization of the nitrates. The decrease in the amount of nitrates was partly the result of the utilization of this form of nitrogen by the bacteria. Qualitative tests for nitrites showed that they were absent from the two check solutions, but the test was very strong in all of the inoculated solutions. This leads to the assumption that the nitrites were produced by the bacteria, either as the first step in the utilization of

the nitrates, or as a by-product. On account of lack of solution it was not possible to make total nitrogen determinaticns in all of the solutions, but equal portions of the duplicate solutions were used. The frequent contamination met with lowered the number of determinations still more, but the results showed a loss of nitrogen from the inoculated flasks.

TABLE 18

Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of potassium nitrate to a liter

(Results expressed as milligram nitrogen to 100 cc. of solution)

ORGANISM	AMMONIA AND AMINO ACID	NITRATES AND NITRITES		TOTAL NITROGEN
		Total	Utilized	
Check	0.32	16.20	None	15.12
Check	0.38	15.08	None	.....
113	0.32	12.27	3.37	14.28
113	1.89	14.80	0.84	.....
130	0.75	9.19	6.45	.....
130*	....	.....	....	.....
131	0.32	10.31	5.33	11.47
131	0.22	10.88	4.76	.....
132	0.43	5.28	10.36	11.47
132	0.27	9.75	5.89	.....
133	1.94	8.91	6.73	.....
133*	....	.....	....	.....
413*	....	.....	....	.....
413*	....	......	....	.....
414	0.92	6.95	8.69	.....
414*	....	.....	....	.....
415	0.49	14.23	1.41	.....
415*	....	.....	....	.....
416*	....	.....	....	.....
416*	....	.....	....	.....

* Culture contaminated.

DISCUSSION

A general study of the data presented shows that the two species of legume bacteria tested were able to utilize some of the nitrogen present in a number of organic and inorganic compounds. During this utilization ammonia was produced from certain of the amino acids in excess of that utilized by the bacteria. The reaction probably was the result of the action of a group of enzymes known as deaminases which activate the hydrolysis of the amino acid, producing ammonia as one of the end products. Whether this preceded the utilization of the nitrogen or whether it occurred later was not definitely proved, although two facts indicate that the latter hypothesis may be the correct one.

In the first place no ammonia was found in the inoculated solutions containing valine, glutamic acid, tyrosine, cystine, amino benzoic acid, or phenylala-

nine. The utilization of some of these compounds was so small that small amounts of ammonia might have been produced and all of this ammonia later utilized, leaving no measurable amount present at the end of the incubation period. However, more of the amino acid nitrogen was utilized from some of the above compounds and at least small amounts of ammonia would probably still be present, if it had been produced.

Secondly, the utilization of the amino nitrogen by the type B strains of *Rhizobium japonicum* growing in glutamic acid solution was so much greater than the utilization of the ammonia furnished by ammonium sulfate that it seems probable, at least in this instance, that the utilization was direct.

Both amino and amido nitrogen were apparently changed in the solutions containing asparagine, but the degree of utilization cannot be arrived at from the determinations which were made. Urea was probably hydrolyzed by the action of urease previous to utilization, for, although no tests were made to determine the actual form utilized, this change seems to have occurred readily in the inoculated solutions.

The nitrogen present in ammonium sulfate was utilized in varying amounts by all of the organisms tested. Whether this was first built up into amino acid and then synthesized into more complex compounds was not determined. The amounts used were usually larger in the solutions inoculated with *Rhizobium japonicum* than in those inoculated with *Rhizobium meliloti*. This may be explained by the fact that the soybean organisms tend to produce an alkaline reaction, which is more suitable than the acid reaction that would occur as a result of the sulfuric acid in the solution following the utilization of the ammonia.

Nitrates were also used in varying amounts by all of the organisms tested. The presence of nitrites in all of the cultures indicates that this may be the first product and that this is then used in the building up of the more complex nitrogenous compounds. The wide variation in the amino and ammonia nitrogen present in the solutions containing nitrates does not allow any conclusions to be dawn regarding their production. The loss of nitrogen may have been the result of a loss of free nitrogen produced during the reduction process which occurred.

Although no tests were started using a nitrite compound as the nitrogen carrier the frequent occurrence of nitrites in the check solutions and their absence in the inoculated solutions indicates that nitrites may be used, at least in small amounts by the legume bacteria.

Inasmuch as ammonia and nitrites have been found to be produced from other nitrogenous compounds by legume bacteria the presence of either of these in the nodules or in solutions which contain these bacteria does not indicate that they are the forms in which the nitrogen is fixed. On the other hand, the ability of the legume bacteria to produce ammonia from a number of compounds does point to the possibility that ammonia may be the product which the plant obtains from the nodule. No attempt was made to test for or obtain bacteroids in these experiments. If these are the forms which are re-

sponsible for fixation, as some writers suggest, it would appear that they are more active physiologically, and thus, it is possible that in this form the changes produced would be of much greater magnitude than those found.

In regard to the value of the tests made as a means of differentiating between species a few points may be emphasized.

There were apparently no differences in the changes produced by the two species in peptone, dl-valine, dl-phenylalanine, p-amino benzoic acid, ammonium sulfate, and potassium nitrate. In certain other compounds such as dl-alanine, l-tyrosine, d-glutamic acid, asparagine, and l-cystine the differences between the two types within the species were as great as the differences between species. However, there were observable differences between *Rhizobium meliloti* and *Rhizobium japonicum* in some of the solutions.

The changes produced in urea were consistently greater in the solutions inoculated with *Rhizobium meliloti* than in those inoculated with *Rhizobium japonicum*. This was very noticeable in the total nitrogen content, the cultures inoculated with alfalfa organisms having lost much more nitrogen than those inoculated with the soybean organisms. If this loss was the result of a loss of ammonia, then the production of ammonia must also have been much greater.

The experiments with glycocoll gave widely differing results. In the first test (table 1) there appeared to be a very significant difference in the ability of the two species studied to produce ammonia and utilize the nitrogen. In the second test, however, no utilization was apparent in any of the solutions. This observed difference may have been due to the increase in the amount of glycocoll added in the second test, making it toxic to both species, whereas the smaller amount used in the first test was toxic only to *Rhizobium japonicum*.

The colors produced in the tyrosine solutions were probably due to the difference in the enzyme action. The action of tyrosinase proceeds in three steps, the first substance produced being red, then this is converted to a colorless compound, which in turn is changed to melanin, a black compound. In acid solutions, tyrosinase changes the tyrosine only partially with the production of a compound having a red color, whereas in alkaline solutions the reaction proceeds farther with the production of melanin. These characteristics then appear to be linked with reaction rather than with the character of the enzyme produced, and can not be considered as being more accurate than measurements of reaction change. The lack of color production in the solutions inoculated with the type B organisms does not prove that the enzyme tyrosinase was absent, but its action may have been intermediate between the alfalfa and type A soybean organisms.

The results obtained with the solutions of dl-amino-n-butyric acid indicate that this amino acid may help in differentiating *Rhizobium meliloti* and *Rhizobium japonicum*. There was a significant production of ammonia and also a marked utilization of amino acid nitrogen in all of the solutions inoculated with the alfalfa bacteria, but only one culture of the soybean bacteria showed any production of ammonia. It is probable, in view of the other results, that this one culture had become contaminated.

By properly regulating the concentration of the amino acid added it appears that any one of the three simple mono-amino-mono-carboxylic acids (glycocoll, alanine, and amino-butyric acid) may aid in differentiating *Rhizobium meliloti* from *Rhizobium japonicum*. There may also be found to be a difference in the value of the dextro- and levo-amino acids which did not appear in the tests, which were, for the most part, conducted with the racemic mixtures of these two.

More striking differences were observed in the action of the types within the species. Type A strain of the alfalfa bacteria utilized much more of the amino acid nitrogen and produced more ammonia from dl-alanine than did the type B strains. Inasmuch as the test for ammonia can be made much more easily than the test for amino acids it may be possible to adapt this test to distinguish between these two types. Type A strains of soybean bacteria did not produce any ammonia nor use any of the amino nitrogen from dl-alanine, but the type B strains were able to make use of some of this nitrogen and to produce small quantities of ammonia. This difference could be made the basis of a test to differentiate these types.

The results obtained by inoculating *Rhizobium meliloti* into solutions containing dl-amino-n-butyric acid are similar to those obtained in alanine solutions. The type A strains apparently used more of the amino acid nitrogen and produced more ammonia than did the type B strains. None of the soybean bacteria tested were able to change appreciable amounts of dl-amino-n-butyric acid.

There were no significant differences in the utilization of d-glutamic acid by the alfalfa bacteria, but such differences were found in the solutions inoculated with *Rhizobium japonicum*. The type B strains of this latter species utilized nearly all of the amino acid nitrogen present, while the type A strains utilized only about one-sixth of the amino nitrogen. There was a considerable difference in the turbidity of the solutions, indicating more rapid growth by the type B strains, and it is quite probable that similar differences in growth would occur on agar slants containing glutamic acid.

Although there appeared to be a slight difference in the utilization of l-cystine by the two types of *Rhizobium meliloti* only one result was obtained for the type A strain, and no conclusions regarding the value of this amino acid in type differentiation can be drawn. There do, however, appear to be differences in the utilization by the different types of soybean organisms, the type B strains using about twice as much of the amino nitrogen as the type A strains.

The utilization of tyrosine was greater by the type B strains of *Rhizobium meliloti* than by the type A strains. The reverse was true in the solutions inoculated with *Rhizobium japonicum*, the type A strains using more of the tyrosine than the type B strains. The differences in color noted in the solutions inoculated with *Rhizobium japonicum* were probably due to a difference in the reaction of the solutions.

The value of p-amino-benzoic acid in the differentiating of types within species

appears not in the utilization of the amino nitrogen but in the production of the dark color in the solutions inoculated with the type B strains of *Rhizobium japonicum*. If this is found to be a constant characteristic of all of the type B strains but not of type A strains it will prove valuable in their separation. Asparagine also appears to have some merits which recommend its use in the differentiation of the two types of soybean bacteria. The production of ammonia was much greater in the solutions inoculated with the type B strains than in those containing the type A strains. Such a difference did not exist between the two types of alfalfa bacteria.

Dl-valine, dl-phenylalanine, urea, ammonium sulfate, and sodium nitrate did not appear to have any value in distinguishing the types in either *Rhizobium meliloti* or *Rhizobium japonicum*.

It is of interest to note that in the case of dl-alanine and l-cystine the utilization of the amino acid nitrogen was greater by the type A alfalfa strain and by the type B soybean strains, and in tyrosine the type B alfalfa strains and the type A soybean strains utilized more of the amino acid nitrogen. In no case was the greatest utilization found in the type A strains of both organisms or in the type B strains of the two. Apparently the type B strains of the alfalfa organisms are more closely related in their nitrogen utilization to the type A soybean strains, whereas the type A strains of alfalfa bacteria are more closely related to the type B soybean organisms.

One other fact deserves mention, namely, the high utilization of d-glutamic acid and asparagine by the type B strains of *Rhizobium japonicum*. These organisms are not as efficient in nitrogen fixation in the plant, and from the foregoing it appears as if this might be because these organisms were better able to utilize the nitrogen present in the plant, and were not forced to depend on the atmospheric nitrogen for their supply of this element.

It appears evident from the data presented here that the legume bacteria even when outside the plant may play some rôle in the nitrogen cycle. However, this is probably not as great as it would appear to be from the aforementioned experiments because of the lack of food material and the competition of other organisms. But they certainly can utilize soluble nitrogenous compounds and under certain conditions may be able to bring about a loss in the total nitrogen content of the medium upon which they are growing.

SUMMARY AND CONCLUSIONS

Studies of the changes produced by *Rhizobium meliloti* and *Rhizobium japonicum* in nitrogenous compounds in solutions were made. The nitrogenous compounds tested were glycocoll, dl-alanine, dl-amino-n-butyric acid, dl-valine, d-glutamic acid, l-cystine, l-tyrosine, dl-phenylalanine, p-amino benzoic acid, urea, asparagine, peptone, ammonium sulfate, and potassium nitrate. Analyses were made for ammonia, nitrates, nitrites, amino acid nitrogen, and total nitrogen.

Changes were noted in the form of nitrogen present in the solutions contain-

ing glycocoll, dl-alanine, dl-amino-n-butyric acid, d-glutamic acid, l-cystine, l-tyrosine, asparagine, urea, ammonium sulfate, and potassium nitrate.

Ammonia was produced by *Rhizobium meliloti* from glycocoll, dl-alanine, dl-amino-n-butyric acid, asparagine, and urea. *Rhizobium japonicum* produced ammonia from dl-alanine, asparagine and urea.

Nitrates were utilized by both of the species tested. All of the inoculated solutions containing nitrates showed an increase in nitrites, which were probably produced during the utilization of the nitrates.

Nitrites were apparently utilized in small amounts by *Rhizobium meliloti* and *Rhizobium japonicum.*

Some of the nitrogen present in the amino group in glycocoll, dl-alanine, dl-amino-n-butyric acid, d-glutamic acid, l-cystine, and l-tyrosine was changed by *Rhizobium meliloti.* Changes in the amino nitrogen in dl-alanine, d-glutamic acid, l-cystine and l-tyrosine were produced by *Rhizobium japonicum.*

Differences in the changes produced by *Rhizobium meliloti* and *Rhizobium japonicum* were noted in glycocoll, l-tyrosine, dl-amino-n-butyric acid, and urea.

Differences were found in the action of the type A and the type B strains of *Rhizobium meliloti* in dl-alanine, dl-amino-n-butyric acid, and l-tyrosine. Differences also appeared in the changes produced by the type A and the type B strains of *Rhizobium japonicum* in dl-alanine, l-tyrosine, d-glutamic acid, l-cystine, p-amino benzoic acid, and asparagine.

REFERENCES

(1) Allison, F. E. 1927 The growth of *Bacillus radicicola* on artificial media containing various plant extracts. *Jour. Agr. Res.* 35: 915–924.

(2) Baldwin, I. L., Fred, E. B., and Hastings, E. G. 1927 Grouping of legumes according to biological reactions of their seed proteins. *Bot. Gaz.* 83: 217–243.

(3) Davisson, B. S., and Parsons, J. T. 1919 The determination of total nitrogen including nitric nitrogen. *Jour. Indus. and Engin. Chem.* 11: 306–311.

(4) Fred, E. B. 1912 A physiological study of the legume bacteria. *Va. Agr. Exp. Sta. Rpt.* 1912: 145–173.

(5) Hills, T. L. 1918 Influence of nitrates on nitrogen-assimilating bacteria. *Jour. Agr. Res.* 12: 183–227.

(6) Leonard, L. T. 1929 Nitrite production by some strains of cowpea and soybean organisms. *Jour. Bact.* 17: 19.

(7) Lewis, L. L., and Nicholson, J. F. 1905 Soil inoculation. Tubercle-forming bacteria of legumes. Okla. Agr. Exp. Sta. Bul. 68.

(8) Müller, A., and Stapp, C. 1926 Beiträge zur Biologie der Leguminosen-knöllchenbakterien mit besonderer Berücksichtigen ihrer Artverschiedenheit. *Arb. Biol. Reichsanst Land u. Forstw.* 14: 455–554.

(9) Stevens, J. W. 1925 A study of various strains of *Bacillus radicicola* from nodules of alfalfa and sweet clover. *Soil Sci.* 20: 45–65.

(10) Van Slyke, D. D. 1911 A method for quantitative determination of aliphatic amino groups. *Jour. Biol. Chem.*, 9: 185–204.

(11) Wright, W. H. 1925 The nodule bacteria of soybeans. I. Bacteriology of strains. *Soil Sci.* 20: 95–129. II. Nitrogen fixation experiments. *Soil Sci.* 20: 131–141.

A METHOD OF APPLICATION DESIGNED TO INSURE PROPER DISTRIBUTION OF FERTILIZERS IN FIELD TRIALS WITH FRUIT TREES

L. R. McKINNON AND OMUND LILLELAND

University of California

Received for publication August 8, 1930

A study of certain soils in the Sacramento Valley in which prune trees suffered a serious scorching of the foliage showed these soils to be distinctly low in both water-soluble and replaceable potassium. The division of plant nutrition in whose laboratories these analyses were made, also reported that these particular soils possessed a high fixing capacity for potassium and predicted that it would be difficult to increase the potassium content to any reasonable depth by the addition of potash salts to the surface of the soil.

Because of the low potash content of these soils and the fact that leaf scorch has been reported as associated with a deficiency of potash, the application of potassium salts in the prune orchards seemed a logical attack in an endeavor to remedy the condition.

A study of the root system of the prune trees in these soils revealed most of the visible roots to be in the second, third, and fourth feet of soil. Even though the trees were relatively shallow rooted the laboratory studies indicated that surface applications of ordinary commercial amounts could not be expected to influence the potash content in the major root zone.

Field studies confirmed the laboratory findings. The results in table 1 indicate that even when 50 pounds of sulfate of potash was broadcast on the surface of the soil over an area of approximately 200 square feet, no increase could be found in the second foot. The application was made in December and the soil samples were taken the following May. The surface 3 inches of soil which composed the dry mulch was discarded in sampling so that it would be more correct to say that no increase in either water-soluble or replaceable potash could be detected below the first 15 inches of soil. In any event the penetration of potash downward was not sufficient to reach many roots and a method had to be devised to overcome this difficulty. This was accomplished with the aid of a power spray rig such as is in use in almost any commercial orchard. The potash salts were dissolved in the spray tank. A specially constructed injection rod made up from ordinary pipe fittings was substituted for the spray rod or gun.

The rod was made of one-quarter-inch gas pipe which was threaded on both ends. The length of this rod depended upon the depth of injection desired. A

hole, one-eighth inch in diameter, was drilled through a plug, which in turn was screwed into a coupling and this in turn onto the one-quarter-inch gas pipe. The plug and coupling were then ground down to a point on an emery wheel. On the other end of the rod was fitted a one-quarter-inch globe valve which takes the standard spray hose nipple. A spade handle connected as indicated in plate 1, figure 1 affords a better grip than is permitted in the original construction. The plug and coupling together with the finished point are also shown.

Generally two such injection rods can be used with the average power spray rig. No difficulty is involved in forcing these into the ground as long as good pressure is maintained. The pressure gauge should register at least 200 pounds with both rods in action.

By the use of this apparatus it has been possible, in the soils mentioned, to increase the potash content to greater depths than a surface application would permit and to place the potash in the root zone. In the experimental field work it was possible to distribute 50 gallons of solution over the 200 square

TABLE 1

Effect of method of application on penetration and distribution of potassium[1]

DEPTH OF SAMPLE	50 POUNDS K_2SO_4 SURFACE		NONE (CHECK)		50 POUNDS K_2SO_4 INJECTED	
	Water soluble K	Replaceable K	Water soluble K	Replaceable K	Water soluble K	Replaceable K
feet	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0–1	9.7	520	0.9	114	4.2	246
1–2	2.1	79	1.1	70	3.2	197
2–3	1.9	41	1.3	44	13.8	592
3–4	2.8	45	1.1	38	3.4	163

feet at the approximate rate of one hole to every square foot to a depth of 3 feet. The time consumed in this operation with two men working was about 15 minutes.

Results of analyses of the soil from the plots which have been injected according to the method noted are shown in table 1. The effectiveness of this type of application in increasing the potash content in the lower depths is evident. The injection rods were generally inserted to a depth of 3 reet and this probably accounts for the greater increase observed at this depth. Much of the potash applied on the surface and unaccounted for in the analysis is undoubtedly fixed in the surface 3 inches which was discarded at the time of sampling. The method has no practical application but meets a particular requirement in experimental work in the field. It assures proper distribution of various salts throughout the soil mass where their effect is being studied on relatively deep-rooted deciduous fruit trees. The fixation of various cations through the

[1] The authors are indebted to P. A. Hibbard, associate chemist in the experiment station, for the analyses reported in table 1.

phenomenon of base exchange and the precipitation of phosphates may be such that surface application of fertilizers may never effect any chemical change in the mass of soil in which the major portion of the tree roots are found. Under these conditions the method of soil injection outlined may be of some assistance to those concerned with soil deficiency studies and fertilizer trials with fruit trees.

PLATE 1

CONSTRUCTION AND OPERATION OF SOIL INJECTION ROD

FIG. 1. Parts used in the construction of the soil injection rod.
FIG. 2. Insuring proper distribution of fertilizer salts with soil injection rods.

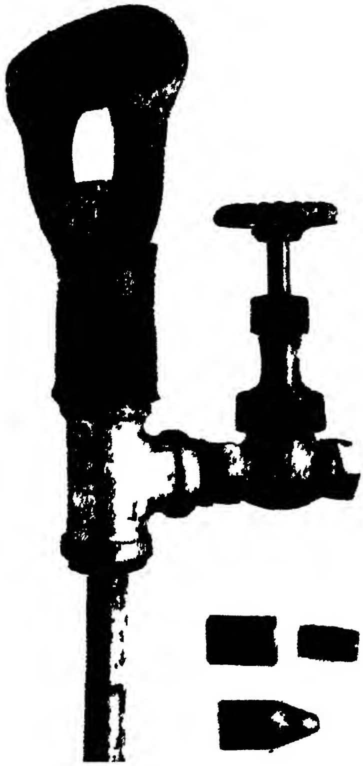


FIG. 1

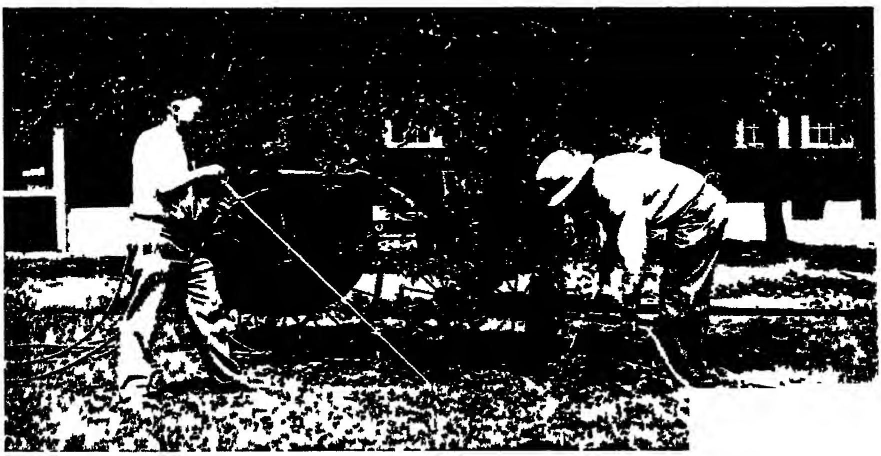


FIG. 2

ERRATA

The fungus flora of the soil, by H. L. Jensen

p. 130—¶4, l. 2, *until* should read *since.*

p. 132—¶3, l. 3, pH *3.3* should read pH *8.3.*

p. 145—Sixth line from bottom, KH_2PO_4, *5* gm., should read KH_2PO_4, *1* gm.

THE CARBON-NITROGEN RATIO IN RELATION TO THE ACCUMULATION OF ORGANIC MATTER IN SOILS[1]

FREDERICK J. SALTER[2]

Ohio State University

Received for publication August 4, 1930

A study of the soil will show that it is a system which is never in a perfect state of equilibrium. It is continually undergoing physical and chemical adjustments with the forces which surround it or are in it. Since these adjustments are never complete, change constantly takes place. One of the most changeable and yet apparently one of the most constant relationships in the soil is that which exists between the soil carbon and the soil nitrogen—the carbon-nitrogen ratio. Many writers have devoted considerable space to organic matter and nitrogen but less has been said in regard to the relation of carbon to nitrogen and the result of various ratios within the soil.

Among the early observations along this line was that regardless of the ratio of carbon to nitrogen in organic materials added to the soil, in a short time the ratio within the soil becomes stabilized at some point near 10 to 1. That this varies somewhat in different localities and at various depths has been shown by several workers. Dyer (14), who gives us one of the earliest observations, studied the carbon-nitrogen ratios of 21 Rothamsted wheat soils at different depths. He found that they varied between 5 and 10 to 1, the surface soils showing a wider ratio than the subsoils. Brown and O'Neal (11) found that, in Iowa, Carrington loam gave a ratio of 12 or 13 to 1, whereas Tama silt loam showed a ratio of 10 to 1. Alway (2) reports that Nebraska soils vary from 11.2 to 13.6:1. Stewart (27) of Illinois states that in general the carbon-nitrogen ratio of the soil tends to become narrower with the aging of the organic material. He found the ratio in the old soils, the gray silt loam, to be 10.4:1; in brown silt loam 12.1:1; and in black clay loam, the younger soil, 11.7:1. Alway and Vail (4) in studying the relative amounts of nitrogen, carbon, and humus in Nebraska soils found that the ratio ran so uniform for surface soils that the carbon content could be estimated with sufficient accuracy from the nitrogen determination. From West Virginia Bulletin 161 it appears that soils of the Huntington series average 13.5:1 carbon-nitrogen ratio whereas those of the Upshur series run 9.8 to 1. The average of the entire state is approximately 11.8 to 1. Ohio soils, from analyses given in by Ames and Gaither (5), average 12 to 1. All of these results indicate that there is some rather stable ratio of carbon to nitrogen for soils of the same region and that the ratio is different in different regions. The factors which most greatly influence the actual ratio are those of climate—temperature and rainfall, the latter as expressed in the ratio R/E, rainfall-evaporation. In regions with a wide rainfall-evaporation ratio and a high temperature there are conditions favorable for luxuriant vegetative growth but at the same time these also favor rapid decomposition processes. Thus, although more organic material may enter into a soil, because of rapid decay it is difficult to keep it.

[1] A thesis presented to the faculty of Ohio State University in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The author is indebted to Dr. Firman E. Bear, formerly head of the soils department, Ohio State University, for many helpful suggestions in connection with this work.

Russell (23) shows from his work on Broadbalk plots that it is only by an increase in carbon that nitrogen can be increased in soils. The reverse is also true that without nitrogen carbon will not be held. Therefore in humid sections the carbon-nitrogen ratio tends to narrow down because energetic decay processes cause a more rapid loss of carbon than of nitrogen. In semi-humid sections, those with a rainfall-evaporation ratio of less than 1, we have conditions less favorable for plant growth and for decomposition. The result is that carbonaceous materials do not disappear as readily as do nitrogenous compounds. This tends to widen the carbon-nitrogen ratio. Jenny (16) points out, from his study of climatic factors as related to soil nitrogen, that the total nitrogen content of soils decreases from North to South in the United States in relation to temperature. In the North, with lower temperature, decay slows down and nitrogen is not so readily lost. This permits of an easier maintenance of organic matter in the soil. In the south on the contrary, high temperatures militate against nitrogen accumulation and thus discourage the keeping of organic matter.

Several workers, notably Alway and McLean (3), Blair and McLean (8), and Sievers and Holtz (25), have pointed out that in soil under cultivation the carbon-nitrogen ratio becomes narrower. Stirring the soil appears to produce a much greater loss of carbon than of nitrogen.

When soils are first brought under cultivation there is a rapid loss of organic matter, but as the amount of nitrogen becomes less the rate of loss slows down and the narrower ratio remains fairly stable.

A number of efforts have been made to associate the carbon-nitrogen ratio with the fertility of the soil. Brown and Allison (10) state, "The determination of the carbon-nitrogen ratio in soils is now coming to be considered of much importance in fertility studies. . . . It shows the rate at which decomposition processes are going on in the soil. Experience has shown that if the ratio narrowed beyond a point of about 10 to 1 crop yields may be reduced evidently because of an insufficient production of available nitrogen, phophorus, and potassium. On the other hand, if the ratio was 12 to 1 or above, bacterial activities apparently occur to a satisfactory extent and sufficient amounts of soluble plant-food are produced for good crop growth." From data presented by Russell (23) it appears that if there is any correlation between productivity and the ratio of carbon to nitrogen, fertile soils have the narrower ratio.

Waynick and Sharp (35) have presented data showing the amount of variation found in the total carbon and total nitrogen of California soils. The samples selected were taken for their apparent uniformity and yet the nitrogen fluctuation was as much as 0.977 to 0.21 and the carbon ran from 1.383 to 0.179. As many as 100 samples were taken from plats of a little over an acre. Since these great variations occurred on small areas which appeared to be uniform these authors discredit any significance that might be given to the association of the carbon-nitrogen ratio with soil fertility. Read (22) has corroborated the work of Waynick and Sharp by the study of samples collected from many experiment stations. His analytical results were compared with the actual yields as reported by the various stations.

Although it seems to be impossible to relate the ratio of carbon to nitrogen in soils to the yields to be expected, this ratio appears to be of considerable importance in determining the processes taking place when organic materials are added to soils.

For a long time farmers have realized that the applications of strawy manure or straw alone have a disastrous effect upon the crop yields immediately following. This may also be the result with some green manures, especially when fairly heavy. Many experiments have been carried out to account for this condition. It was soon recognized that the crop failures were due to a lack of available nitrogen. Lyon, Bizzell, and Wilson (18) found that the dried roots of a number of plants brought about nitrate depression. Hutchison (15) found that applications of carbohydrates to soils caused a decrease in soil nitrates. An application of hay dust and straw at once lowered the amounts of ammonia and nitrate, but after several months these became greater. Murray (21), Scott (24), Collison and Conn (12), and others (1, 9, 19, 38), have obtained harmful effects and the depression of nitrates following the use of straw or other cellulosic materials. The early explanation for this decrease in available

nitrogen was that denitrification took place with increased rapidity due to these microörganisms being greatly favored by the material added. This theory was soon replaced by the belief that soil organisms became consumers of nitrogen under certain circumstances and thereby lessened the stock of nitrates. Murray (21) showed that the total nitrogen content of the soil remains the same, following the disappearance of the nitrates, as it was before, indicating a mere change of form. Doryland (13) has probably carried out the most extensive work to show that soil microörganisms may become competitors to crops for the supply of available plant nutrients. He has shown that these competitors are not necessarily "detrimental" organisms, but may be some of the most vigorous ammonifiers. He points out that the most favorable conditions for the consumption of ammonia or nitrates is an application of "energy material," such as carbohydrates or straw. This use of nitrogen by the organisms is simply a normal metabolic process. For the construction of their body tissue they require a certain amount of nitrogen for each unit of carbon consumed. When a substance having a wide energy-nitrogen ratio is added to the soil, since there is an abundance of energy material but very little nitrogen, the organisms make use of the available nitrogen from the soil in their growth processes. Since the soil organisms are more advantageously situated with respect to this nitrogen, the plant suffers.

The work of Brown and Allison (10) agrees with that of Doryland. An application of narrow-ratio materials, such as rotted manure and legume hays, caused increased yields of oats whereas wide-ratio substances lowered the yields.

That the availability of nitrogen following the turning into the soil of organic materials is probably a matter of the carbon-nitrogen ratio is shown by the work of Blair and Prince (9), Wilson and Wilson (38), Thomas and Harper (28) and others. When straws and other residues with a wide ratio are supplemented with legumes or other sources of nitrogen in order to narrow the ratio, no depression of available nitrogen results. If the ratio is sufficiently narrow some nitrate nitrogen is made available. This is shown in the work of Thomas and Harper (28) using straw alone and also supplemented with sources of nitrogen, such as legume hays, nitrate of soda, and sulfate of ammonia. The combination applications brought about larger amounts of available nitrates, because of a narrowing of the carbon-nitrogen ratio.

Waksman (30) has made a thorough study of the microbiological processes connected with the decomposition of organic materials within the soil. He points out that, added to the soil, organic substances are attacked by many forms of microörganisms, including bacteria, actinomycetes, fungi, and possibly protozoa. As a result of these activities the organic tissues are broken down, the available energy is utilized by the organisms, and the inorganic compounds of nitrogen, phosphorus, and potassium are made available for plant growth. Most of the soil microörganisms, with the exception of algae and some autotrophic bacteria, require organic materials as a source of energy. Therefore these substances must become energy sources for soil organisms. The speed with which the materials are decomposed varies considerably. It is not the organic material as a whole that is attacked, but various constituents of this material. Some of these are rapidly attacked whereas others are affected only slightly. Therefore the nature of the composition of the materials added to soils will determine the speed of its decay and the final result of the decomposition. Since in their metabolic processes the organisms synthesize considerable cell substance, and this requires nitrogen and phosphorus, the speed of decomposition will also be governed by the available supply of these elements.

It has been noted by Whiting (36), Whiting and Schoonover (37), Martin (19), Dvorak [cited by Waksman (31)], Waksman (34, 32) and others, that immature green plant materials are decomposed most rapidly, largely because they contain an abundance of water-soluble carbohydrates and nitrogenous compounds. The leaves of plants decompose more rapidly than their stems or roots, because of their higher nitrogen content. As plants become older their carbonaceous materials become transformed to an increasing extent into cellulose and lignins. These latter are decomposed only very slowly.

That the supply of easily available nitrogen is also important in determining the rate of decomposition has been shown by Waksman (31), Waksman and Starkey (33), Anderson (6), Barthel and Bengtsson (7), Merkle (20) and Starkey (26). Materials that are poor in nitrogen but rich in cellulose decompose more slowly than those rich in nitrogen and poor in cellulose. This may be put in other words—materials with a wide carbon-nitrogen ratio decompose more slowly than those with a narrow ratio. In the case of the former, such as straws, corn stover, wood products, and cellulose, the available supply of nitrogen often may become the limiting factor in their decay. Anderson (6) has shown that the rate of cellulose decomposition increases with the increase in available nitrogen until there is sufficient nitrogen for the maximum growth of the microörganisms. He also found that nitrification could take place when cellulose was present, but no nitrates were found until the needs of the bacteria were met. Barthel and Bengtsson (7) also obtained results similar to those of Anderson. They attributed the favorable influence of stable manure upon the decomposition of cellulose in the soil to the available nitrogen present in the manure. When the quantities of manure were doubled the amount of cellulose decomposed was increased proportionately, and halving the amount likewise decreased the decay. Since the same results were obtained with sterilized manure they concluded that the microörganisms present in the manure have no influence in normal soils. They obtained practically the same results with equivalent amounts of ammonium sulfate and ammonium nitrate. Waksman and Tenney (34), working with rye straw, corn stalks, oak leaves, and alfalfa tops, found that the addition of available nitrogen very greatly increased the rate of decay with all the materials except alfalfa. In the latter case adding nitrogen had no effect, as the nitrogen present in the alfalfa was sufficient to bring about a very rapid decay. Thus it appears that narrowing the ratio of carbon to nitrogen causes an increased rate of decomposition with materials having a wide carbon-nitrogen ratio.

Waksman (29) shows that microörganisms acting in the soil upon added organic materials bring about (*a*) the complete decomposition of much of the organic materials, including most of the carbohydrates and proteins; (*b*) the accumulation of such resistant materials as lignins; (*c*) the reassimilation of part of the carbon and part or all of the nitrogen to form cell protoplasm. The extent to which this latter process goes on depends upon the organisms acting. "If fungi are the active agents of decomposition, one part of nitrogen will be assimilated for every seven or eight parts of carbon given off as CO_2 in the process of energy utilization. If pure cultures of bacteria are the active agents, one part of nitrogen will be assimilated for 12.5 parts of carbon liberated CO_2. Since bacterial cells are richer in nitrogen than are fungus mycelium, a smaller amount of protoplasm will be synthesized. If the mixed soil flora and fauna take part in the decomposition process, one part of nitrogen will be transformed from an inorganic to an organic form for every 16 to 18 parts of carbon given off." This latter process is what ordinarily takes place in the soil. When plant residues or straw is added to the soil in a very short time 60 to 80 per cent of the carbon compounds is decomposed, leaving the remainder in the soil as resistant celluloses, waxes, or lignins. Of this 60 to 80 per cent acted upon, 20 to 70 per cent is given off as CO_2 and 10 to 40 per cent is left in the soil as microbial protoplasm. This indicates that a considerable quantity of the carbon added to a soil in organic materials may be held in the soil as synthesized cell substance. This will contribute to the supply of organic matter or "humus."

The amount of nitrogen utilized by soil microörganisms also varies considerably. In the case of fungi, for every 100 parts of organic matter decomposed, from 0.5 to 4.0 parts of nitrogen will be assimilated. If the organic material contains 0.5 per cent nitrogen, as in the case of straw, additional nitrogen has to be utilized, 1 to 2 parts for every 100 parts of material before complete decomposition takes place. If, however, the added material is clover or alfalfa, containing 1.5 to 2.5 per cent nitrogen, no additional nitrogen is necessary for decay, but no nitrogen will be liberated as NH_3 for a few months. If the proportion of nitrogen runs higher than 2.5 per cent then some of it will at once become available as ammonia.

Bacteria and actinomycetes are not as economical in their utilization of energy materials as fungi, and although their nitrogen content is higher (9 to 13 per cent) the actual amount of nitrogen assimilated is less than in the case of fungi, since they synthesize so much less cell protoplasm. This will result in the liberation of available ammonia from materials having a lower percentage of nitrogen.

In summarizing, the processes carried on by the soil microörganisms result, with materials having a wide carbon-nitrogen ratio, in the use of available soil nitrates, the loss of considerable carbon, and a narrowing of the ratio. If, however, the added substance has a narrow ratio this favors the synthesizing of more cell protoplasm, because of the abundance of nitrogen, with the result that less carbon will be lost, and at the same time nitrogen will be liberated as available nitrates. The result will be a widening of the ratio. A soil having a carbon-nitrogen ratio wider than normal is not in a condition to support plant growth, since the activity of the soil organisms will result in nitrogen starvation. When a soil shows a constant ratio this indicates that there is a proper balance of nitrogen and carbon to meet the needs of the metabolic processes of the soil flora. Some energy material will be utilized and at the same time some nitrogen will be liberated as ammonia. The addition of fresh organic materials having a carbon-nitrogen ratio of 10:1 will result in stimulating the growth of the organisms, the liberation of some CO_2 and the formation of an equivalent amount of ammonia.

EXPERIMENTAL

In none of the investigations cited has there been any work conducted to determine the conditions under which the most organic matter, when introduced in various forms, is retained by the soil. It is evident from the foregoing discussion that the carbon-nitrogen ratio of the materials as well as that of the soil is intimately related to the processes taking place. It was with the purpose of determining the effects of different carbon-nitrogen ratios upon the accumulation of organic matter that these experiments were undertaken.

The soil used was an Ellsworth silt loam, low in carbonates and in organic matter. The original composition was as follows:

Carbon	0.97 per cent
Nitrogen (Kjeldahl)	0.073 per cent
Nitrate nitrogen	6.3 pounds an acre
Phosphorus	0.053 per cent
Potassium	1.40 per cent
pH	4.6
Jones lime requirement	6,270 pounds
Carbon-nitrogen ratio	13.3 to 1

As the pH of the soil was low it was feared that the acidity might inhibit biological activities. Fresh hydrated lime was added at the rate of 4,000 pounds an acre (2,000,000 pounds of soil) at the beginning of the experiment to reduce the acidity to a pH of about 6.0.

As the phosphorus content of this soil was also low it was thought best to prevent its becoming a limiting factor by adding a plentiful supply at the beginning. Superphosphate (20 per cent) was applied at the rate of 1 ton an acre to all cultures with the exception of the blank tests and the soil check.

The suppliers of carbon were employed in such amounts as to add 4,000

pounds of carbon an acre. This was equivalent to adding about 7,000 pounds of humus if the entire amount remained. The organic materials used were corn stover, straw, bluegrass (dry), ragweeds, tobacco stems, wood fiber (sawdust), red clover hay, sweet clover hay, glucose, and cellulose. The composition of these substances is given in table 1. These materials were dried and finely ground to permit a more even distribution in the soil.

The nitrogen supplier used was ammonium nitrate. This was selected because of the belief that it would leave no residue likely to alter the reaction. It was ground fine and added in amounts necessary to adjust the ratios to the desired values.

The ratios selected were 15:1, 10:1, 5:1, and 1:1. It was expected that the 15:1 ratio would be sufficiently wide to promote the loss of considerable carbon and to stimulate some nitrogen fixation. The 10:1 ratio was chosen to typify the normal soil ratio. With this initial ratio it was expected that

TABLE 1

Composition of organic materials used

	CARBON	NITROGEN	C:N RATIO
	per cent	*per cent*	
Corn stover	40.2	0.84	47.8:1
Straw	38.4	0.93	41.3:1
Wood fiber	43.2	0.10	432.0:1
Bluegrass	34.0	1.66	20.5:1
Ragweeds	37.4	3.08	12.0:1
Tobacco stems	34.7	2.02	17.0:1
Red clover	39.7	1.54	26.0:1
Sweet clover	38.6	3.22	12.0:1
Glucose	36.3	0.00	......
Cellulose	44.0	0.00	......

the relation of carbon to nitrogen would remain fairly stable with the contribution of a moderate amount of organic matter and the liberating of some available nitrogen. The 5:1 and the 1:1 ratios, being narrow, were expected to cause considerable organic matter to accumulate and also much ammonia or nitrates to be liberated. With this wide variation in ratios a considerable difference in the rates of decomposition was also anticipated.

Some investigators have reported that additions of phosphates to soils in liberal amounts may bring about increased nitrogen fixation. If such were the case the addition of phosphates to the soil should result in less nitrate depression and a larger accumulation of organic matter. To one culture pot for each of the organic materials phosphate only was added at the rate of one ton an acre.

One series of cultures was also included in which all organic materials were incorporated with the limed soil without supplementary additions of ammonium nitrate. These cultures are designated as "blanks."

As a check upon the process taking place in the soil due to liming, four cultures weie arranged to which no organic materials were added.

One-half gallon glazed crocks were employed. Each of these was filled with 2.5 pounds of soil to which had been added the proper amounts of organic material and the necessary supply of ammonium nitrate for adjusting the carbon-nitrogen ratio to the desired values. The entire amount of soil used had previously been treated with the hydrated lime and the superphosphate. These culture pots were then brought to optimum moisture content (approximately 24 per cent) with distilled water and kept in a room the temperature

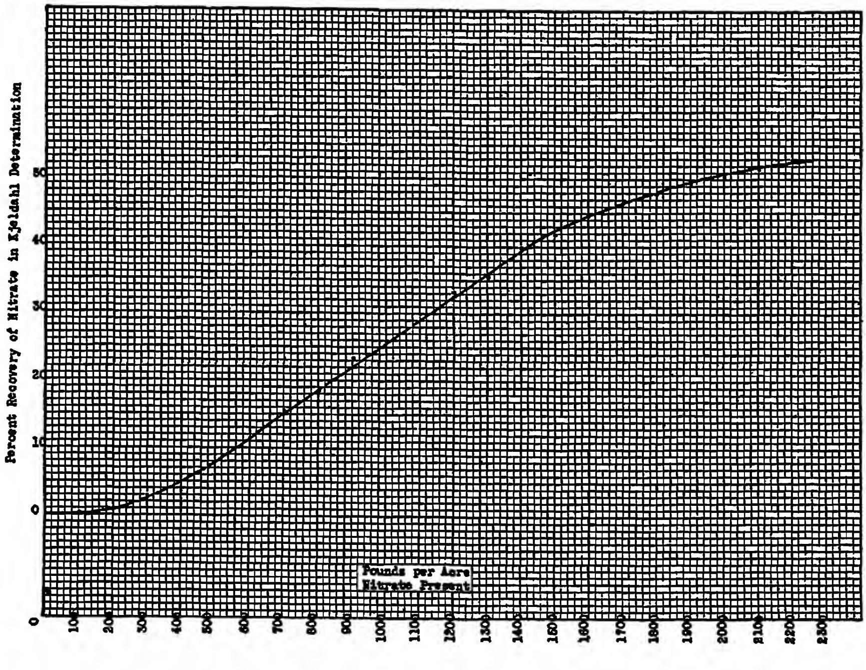


FIG. 1. RELATION OF NITRATE RECOVERY IN THE KJELDAHL METHOD TO THE QUANTITY OF NITRATE PRESENT

and humidity of which was controlled. The temperature was held between 25 and 30°C. The moisture content was adjusted every two weeks, this being frequent enough to limit the loss in water to less than 5 per cent. Samples for analysis were taken at the end of the first, second, fourth, sixth, eighth, and twelfth months. The samples were removed by means of a large cork borer pushed to the bottom of the crocks. The amount removed at each sampling period was about 50 gm. After each sampling the soil was taken out and thoroughly mixed, then replaced. This served to produce good aeration. The samples were dried, ground to pass a 100-mesh screen, and stored in tightly stoppered bottles.

TABLE 2

Organic nitrogen (to each 2,000,000 pounds of soil)

MATERIALS	15:1			10:1			5.1			1:1			PHOSPHATED			BLANKS		
	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month
	pound	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*	*pound*
Corn stover	1,600	1,860	1,850	1,860	1,910	1,890	2,020	1,920	1,885	3,340	3,115	2,580	1,520	1,680	1,430	1,460	1,600	1,300
Straw	.....	.....	.....	1,995	1,800	1,800	2,220	1,855	1,840	3,110	2,910	2,620	1,560	1,620	1,610	1,540	1,620	1,500
Wood fiber	1,620	1,780	1,820	1,790	1,740	1,680	2,115	1,910	1,750	3,110	3,210	3,010	1,600	1,515	1,425	1,480	1,520	1,405
Bluegrass	1,875	1,690	1,780	1,940	1,760	1,860	2,220	1,920	1,890	3,400	3,210	3,080	1,720	1,650	1,540	1,600	1,615	1,555
Ragweeds	.....	.....	.....	1,830	1,880	1,780	2,200	2,040	1,830	3,310	3,300	2,340	1,860	1,860	1,860	1,800	1,855	1,815
Tobacco stems	1,780	1,760	1,780	1,835	1,880	1,815	2,060	1,860	1,860	3,070	2,990	2.930	1,640	1,630	1,600	1,620	1,720	1,675
Red clover	1,660	1,675	1,800	1,780	1,920	1,920	2,060	1,880	1,760	3,460	2,980	2,340	1,660	1,650	1,680	1,620	1,635	1,630
Sweet clover	.....	.....	.....	1,940	1,800	1,800	2,460	2,020	1,850	3,200	3,200	3,230	1,900	1,875	1,720	1,880	1,800	1,800
Glucose	1,755	1,640	1,690	1,785	1,775	1,715	2,020	1,780	1,850	2,980	3,090	2,800	1,440	1,595	1,495	1,420	1.590	1,515
Cellulose	1,760	1,640	1,640	1,740	1,860	1,825	2,135	1,890	1,810	3,040	2,780	2,740	1,520	1,560	1,495	1.480	1,500	1,440
Average	1,720	1,720	1,765	1,850	1,830	1,810	2,150	1,910	1,835	3,200	3,080	2,770	.....	.....	.....	.....	.....	.....
Soil check	1,420	1,480	1,300	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

The results for the 2-, 4-, and 8-month periods are omitted for the purpose of simplification.

TABLE 3

Organic carbon (for pounds to each 2,000,000 pounds of soil multiply results by 1,000)*

MATERIALS	15:1			10:1			5:1			1:1			PHOSPHATED			BLANKS		
	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month
Corn stover	19.91	18.54	18.34	20.86	18.72	18.49	20.67	18.71	18.56	20.75	19.20	18.90	20.59	18.14	18.00	20.67	18.49	17.05
Straw	....	....	....	19.93	19.20	18.54	19.96	18.87	18.63	20.89	19.50	18.73	20.10	18.30	18.35	20.45	18.27	17.37
Wood fiber	20.86	19.09	18.27	20.67	19.22	18.68	20.34	19.36	18.76	20.72	19.41	18.95	20.94	18.50	17.23	21.05	19.80	16.90
Bluegrass	20.78	19.17	18.73	20.86	18.35	18.54	20.20	19.09	18.98	22.09	19.81	19.23	20.86	18.95	17.78	20.45	19.22	18.13
Ragweeds	....	....	....	21.32	18.81	18.27	20.56	19.14	18.54	20.40	19.03	18.65	20.45	18.50	18.00	21.70	19.77	18.73
Tobacco stems	21.13	18.95	18.40	20.61	19.03	18.68	20.86	19.36	18.81	20.72	19.05	18.87	20.20	18.10	17.89	21.54	18.68	18.40
Red clover	20.45	18.32	18.32	20.31	18.84	18.43	19.36	19.00	18.84	20.72	18.46	18.40	19.14	18.19	17.75	19.30	18.40	18.19
Sweet clover	....	....	....	20.45	19.28	18.54	21.00	18.65	18.76	19.50	18.90	18.92	20.12	18.35	18.13	20.89	18.43	18.38
Glucose	19.74	17.48	17.31	20.18	17.37	17.02	19.55	17.72	17.04	19.71	17.67	16.23	18.46	17.15	15.87	18.73	17.45	16.44
Cellulose	18.84	17.40	17.42	18.87	17.89	17.73	19.14	17.72	18.00	19.63	18.43	18.05	20.26	17.18	16.20	21.40	17.50	17.50
Average	20.24	18.42	18.11	20.41	18.67	18.29	20.16	18.76	18.49	20.51	18 95	18.49	....	....	....	....	....	....
Soil check	17.72	16.63	16.36	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

* Carbon reported includes negligible amounts of inorganic carbon.

TABLE 4

Ratios of carbon to nitrogen

MATERIALS	15:1			10:1			5:1			1:1			INITIAL RATIO	PHOSPHATED			BLANKS		
	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month		1st month	6th month	12th month	1st month	6th month	12th month
Corn stover	12.4	9.9	9.8	11.1	9.8	9.8	10.4	9.7	9.9	6.2	6.2	7.3	15.5	13.5	10.8	12.6	14.1	11.5	13.0
Straw	.....	.....	.....	10.0	10.7	10.3	9.0	10.1	9.5	6.7	6.7	7.2	15.0	12.9	11.3	11.4	13.3	11.3	11.5
Wood fiber	12.9	10.7	10.0	11.5	11.0	11.1	9.6	10.1	10.7	6.1	6.1	6.2	16.1	13.1	12.2	12.0	14.2	13.0	12.0
Bluegrass	11.1	11.3	10.5	10.7	10.4	10.0	9.1	9.9	10.0	6.5	6.0	6.2	14.1	12.1	11.5	11.5	12.7	11.9	11.7
Ragweeds	.....	.....	.....	11.6	10.0	10.2	9.3	9.4	10.1	6.2	5.8	8.0	13.1	11.0	10.0	9.7	12.0	10.6	10.3
Tobacco stems	11.8	10.7	10.3	11.2	10.1	10.2	10.1	10.4	10.1	6.9	6.4	6.4	13.8	12.3	11.6	11.2	13.3	10.8	11.6
Red clover	12.3	10.9	10.2	11.4	9.8	9.6	9.4	10.1	10.6	6.0	6.2	8.0	14.5	11.5	11.0	10.5	11.9	11.2	11.2
Sweet clover	.....	.....	.....	10.5	10.7	10.3	8.5	9.2	10.1	6.1	5.9	5.9	13.0	10.6	9.7	10.5	11.1	10.2	10.1
Glucose	11.3	10.6	10.2	11.3	9.8	9.8	9.7	9.9	9.2	6.6	5.7	6.0	16.1	12.8	10.7	10.6	13.2	11.0	10.8
Cellulose	10.7	10.6	10.6	10.8	9.6	9.7	9.0	9.4	9.9	6.4	6.7	6.5	16.1	13.3	10.8	10.8	14.4	11.6	12.1
Average	11.8	10.7	10.2	11.0	10.2	10.1	9.4	9.8	10.0	6.4	6.2	6.8	.....	.....	.....	.....	.....	.....	.....
	Initial ratio	1st month	6th month	12th month															
Soil check	13.3	12.5	11.2	12.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE 5

Nitrate nitrogen (to each 2,000,000 pounds of soil)

MATERIALS	15:1				10:1				5:1				1:1				PHOSPHATED			BLANKS		
	At start	1st month	6th month	12th month	At start	1st month	6th month	12th month	At start	1st month	6th month	12th month	At start	1st month	6th month	12th month	1st month	6th month	12th month	1st month	6th month	12th month
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
Corn stover	95	50	120	440	160	110	160	440	360	440	560	520	1,960	1,540	1,920	2,400	50	140	180	40	120	120
Straw	85	...	...	...	150	160	520	560	350	480	640	580	1,950	1,920	1,920	2,160	40	140	200	60	120	80
Wood fiber	135	180	320	320	200	200	520	320	400	360	640	660	2,000	1,920	1,440	1,920	30	160	240	30	120	280
Bluegrass	40	160	440	280	105	320	320	120	305	480	640	480	1,905	1,760	1,760	1,600	60	220	280	60	180	260
Ragweeds	...	...	...	...	35	220	320	360	235	280	520	640	1,835	1,540	1,840	2,560	120	360	320	140	400	320
Tobacco stems	20	100	320	320	85	200	560	400	285	320	720	560	1,885	1,760	1,920	1,760	70	440	320	110	360	400
Red clover	60	50	400	480	125	50	440	440	325	280	640	700	1,925	1,020	1,960	2,240	60	220	320	70	160	240
Sweet clover	..	...	...	...	35	100	480	640	235	480	680	640	1,835	1,720	1,760	1,800	120	360	320	120	320	360
Glucose	135	200	280	320	200	260	400	400	400	280	560	540	2,000	1,760	1,920	2,240	60	220	180	60	240	200
Cellulose	135	280	280	320	200	300	320	240	400	320	520	600	2,000	1,760	1,760	2,400	50	80	200	40	100	240
Average	...	146	308	354	...	192	404	392	...	372	612	592	...	1,670	1,820	2,108	....	....	....	....	....	....
Soil check	...	100	180	100	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

Nitrogen was determined by the Kjeldahl method and nitrate nitrogen was measured by the phenoldisulfonic acid method. In order to determine the extent to which nitrate nitrogen was included in the Kjeldahl determination a considerable number of total nitrogen determinations were made by a modification of the Davisson method.[3] This method requires so much time that it does not lend itself to large numbers of determinations. It was found, however, that the fractional recovery of nitrate nitrogen in the standard Kjeldahl method was a function of the total amount of nitrates present and that by the application of a correction factor, total nitrogen including nitrates

TABLE 6

Gain or loss in total nitrogen at end of twelfth month (by each 2,000,000 pounds of soil)

MATERIALS	15:1	10:1	5:1	1:1	PHOSPHATED	BLANKS
	pounds	*pounds*	*ponnds*	*pounds*	*pounds*	*pounds*
Corn stover	+560	+470	+145	−480	+110	−120
Straw	.....	+500	+160	−580	+250	+20
Wood fiber	+410	+140	+150	−530	+205	+225
Bluegrass	+330	+120	+110	−780	+165	+160
Ragweeds	.....	+280	+210	−560	+390	+345
Tobacco stems	+370	+350	+160	−770	+230	+385
Red clover	+550	+500	+200	−880	+390	+260
Sweet clover	.....	+580	+230	−430	+250	+370
Glucose	+280	+250	+130	−420	+215	+255
Cellulose	+230	+200	+150	−320	+235	+220
Average	+390	+339	+165	−575	.....	.....

could be estimated with fair accuracy from the results of the Kjeldahl determination.

Table 2 shows the corrected amounts of organic nitrogen[4] to the acre.

The electric combustion method was employed for determining organic carbon (table 3)

The ratios of carbon to nitrogen, calculated from the foregoing carbon and nitrogen values, are presented in table 4.

The quantities of nitrate nitrogen found at successive periods are presented in table 5.

By calculating the total amount of nitrogen present in the soil, the organic

[3] The original Davisson method consists in reducing the nitrates with Devarda's alloy under slightly alkaline conditions. It employs 0.1 N/NaOH solution and boiling for 20 minutes. This did not give complete recovery with known solutions, therefore 0.25 N/NaOH solution was substituted and the mixture permitted to stand in the cold over night. After acidifying with 35 cc. of H_2SO_4 and evaporating off the water the regular Kjeldahl procedure was followed. The graph constructed from the results of these determinations and used to facilitate the calculations of the amounts of organic nitrogen is given as figure 1.

[4] Organic nitrogen includes any ammonia nitrogen remaining unnitrified from that originally supplied as ammonium nitrate.

nitrogen plus the nitrate nitrogen, and from this subtracting the nitrogen present at the beginning of the year, that nitrogen added by fixation or lost by denitrification can be obtained for any period. The results for the12-month period are shown in table 6.

DISCUSSION

The principal purpose of this investigation was to determine the influence of the carbon-nitrogen ratio of organic additions upon the accumulation of organic matter in the soil, as estimated from organic carbon. The amounts of carbon (table 3) show that regardless of the sources of the carbon the quantities present at the end of the year fall within a rather narrow range. It is perhaps to be expected that different materials adjusted to a common ratio would show little variation except possibly in the rapidity of change. Such seems to be the case.

The 15:1 ratio has resulted in the accumulation of least carbon as measured after 12 months incubation. Such a ratio evidently encourages the evolution of much carbon as carbon dioxide, the energy material being in excess of the supply of available nitrogen. For each material the amount of carbon remaining is less than with the narrower ratios. Thus it appears that a wide ratio is not conducive to the best accumulation of organic matter. With this wide ratio there should be a tendency for nitrogen fixation to occur. Krishna (17) has recently shown that straw and other carbonaceous materials can furnish energy for nitrogen fixation. Table 6 shows that fixation has occurred with all materials, although to the greatest extent with the more carbonaceous. With corn stover, wood fiber, and red clover the organic nitrogen content increased from approximately 1,600 to over 1,800 pounds an acre by the end of the year. When soils are treated with wide ratio materials there is a tendency for the ratio to become narrower as a result of the loss of carbon and the fixation of nitrogen. This is indicated in table 4. From an initial ratio of 15 to 1, soils treated with corn stover narrowed to 9.8 to 1, and those treated with red clover to 10.2 to 1. Other treatments showed similar results.

The 10 to 1 ratio was thought to represent the stable relation for soils of the region. That such is the case is shown by the amounts of organic carbon and nitrogen remaining in the soils for those cultures having this initial ratio. While there was an evolution of some carbon as carbon dioxide there was an equivalent release of nitrogen as nitrates or otherwise. This resulted in the ratio of carbon to nitrogen remaining practically stable. Table 4 indicates that at the beginning of the year nitrogen was lost more rapidly than organic carbon, resulting in a widening of the ratios. But, after a short time the carbon losses were sufficiently large so that the ratio remained approximately 10 to 1.

The 5:1 and 1:1 ratios have resulted in the largest storage of organic carbon (table 3). This is in accord with the expectation that a narrow ratio should encourage the holding of carbon and the release of nitrogen.

Figure 2 shows the trend of the various ratios plotted from the averages. It indicates the tendency to stabilize at the 10 to 1 ratio. That a narrow ratio of carbon to nitrogen favors the loss of nitrogen is shown in table 6. From the amounts of organic nitrogen in the materials it is seen (table 2) that a considerable loss occurred in every case with the narrow ratios. The rate of loss of nitrogen was much greater than the decrease in organic carbon, this causing a widening of the carbon-nitrogen ratio, as shown in table 4. The 5 to 1 ratios have widened in all cases, the final results ranging from 9.2 to 10.7:1. The 1 to 1 ratio has also spread considerably, ending at values ranging from 6.0 to 8.0:1.

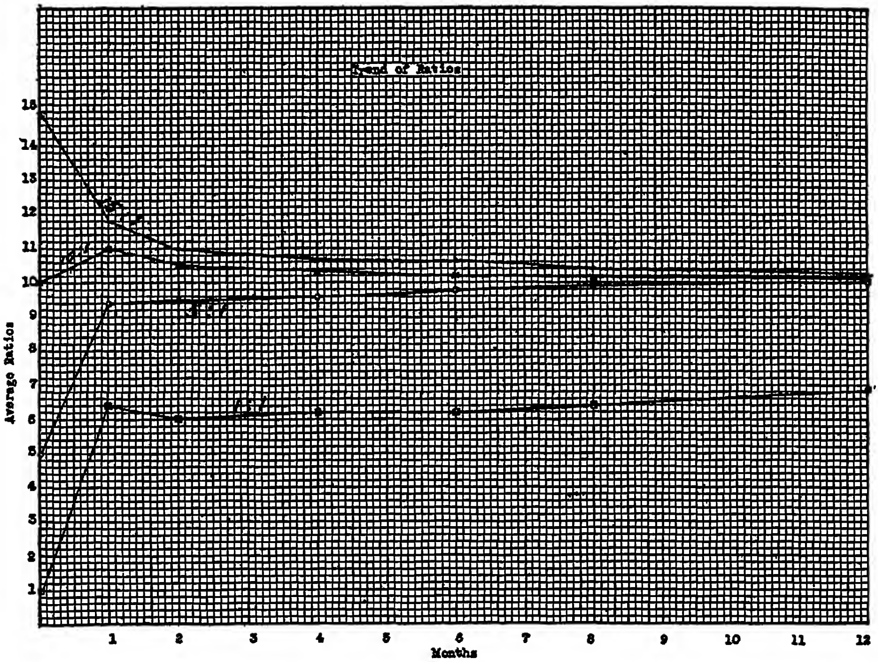


FIG. 2. TREND OF CARBON TO NITROGEN RATIOS

The availability of the nitrogen as measured by the nitrate production varied greatly with the different ratios (table 5). From the analyses reported it is apparent that the 15:1 ratio at no time furnished much available nitrogen. During the first months several of the organic materials, (Blanks), corn stover, tobacco stems, and red clover caused a marked nitrate reduction. The 10 to 1 ratio cultures show a larger production of nitrates. This is probably because the supply of nitrogen meets the needs of the microörganisms and nitrogen is liberated in proportion as carbon is utilized as a source of energy. The largest amounts of nitrates present are found with the 5 to 1 and the 1 to 1 ratios. The quantities amount to as much as 2,500 pounds an acre. These results are in

accord with the findings of Waksman (31) and of Lyon, Bizzell, and Wilson (18). They indicate that with nitrogen in excess of the energy supply nitrogenous materials will be utilized as a source of energy and nitrogen will be liberated as ammonia, later being nitrified to nitrates.

Waksman and Tenney (34), Whiting (36), and others have shown that the rate of decomposition of organic materials varies directly with the amounts of soluble constituents and of available nitrogen. Those materials having the larger amounts of these components will undergo decay most rapidly. Tables 2 and 3 show that there is a difference in the rates at which these organic materials have undergone decay. During the first month the most rapid action took place with the wide-ratio materials. Waksman points out that, with readily available nitrogen supplied, many highly carbonaceous materials will decay very rapidly because of their high content of cold-water soluble constituents. This probably was the case with these organic substances. By the end of the second month, however, the most readily available components

TABLE 7

Order of rapidity of decomposition (most rapid at top)

FIRST MONTH	SECOND MONTH	FOURTH MONTH	SIXTH MONTH	EIGHTH MONTH	TWELFTH MONTH
Straw	Ragweeds	Sweet clover	Sweet clover	Sweet clover	Sweet clover
Corn stover	Sweet clover	Red clover	Red clover	Red clover	Ragweeds
Red clover	Red clover	Corn stover	Corn stover	Ragweeds	Red clover
Bluegrass	Corn stover	Ragweeds	Ragweeds	Corn stover	Straw
Wood fiber	Straw	Bluegrass	Tobacco stem	Wood fiber	Corn stover
Tobacco stem	Wood fiber	Straw	Blue grass	Straw	Wood fiber
Sweet clover	Bluegrass	Wood fiber	Straw	Tobacco stem	Tobacco stem
Ragweeds	Tobacco stem	Tobacco stem	Wood fiber	Bluegrass	Bluegrass

of these wide-ratio materials probably were largely decomposed, since from this time on, the narrow ratio materials showed the most rapid rate of change. The order of the rates of decomposition, as determined from the data for organic carbon and organic nitrogen, considering the losses in each interval of time, is given in table 7.

The results obtained with the blank cultures (table 8) show that at the end of the year the amounts of organic carbon remaining in the soil are directly in accord with the carbon-nitrogen ratios. Ragweeds, sweet clover, and tobacco stems, those materials having the narrower ratios, have resulted in the accumulation of most organic matter. The artificial materials, glucose and cellulose, have shown large losses of carbon. These results support the statement of Russell (23) that to increase carbon in a soil it is necessary also to increase the nitrogen supply. Wide-ratio materials containing little nitrogen do not favor carbon accumulation.

The depression of nitrates characteristic of applications of straw and other wide-ratio materials is noticeable in the blank test cultures (table 5). In the

check soil the quantity of nitrate nitrogen at the end of the first month was 100 pounds an acre. This increased slightly during the year. When wood fiber, straw, bluegrass, corn stover, and other materials low in nitrogen were added to the soil the quantity of nitrate nitrogen was reduced to 30 to 40 pounds an acre, indicating considerable nitrate consumption. In some cases the depression of the nitrates was manifest during most of the 12-month period. The only materials which did not reduce nitrate nitrogen were those having a carbon-nitrogen ratio lower than 20:1.

Nitrogen fixation is also observed to have taken place with the blank tests. Table 6 shows that at the end of the year some fixation had occurred in all cases except with applications of straw and corn stover. The largest amount

TABLE 8

Summary of blank cultures

MATERIAL	INITIAL RATIO	ORGANIC CARBON*				ORGANIC NITROGEN			
		Orig-inal	12th month	Loss	Gain over check	Orig-inal	12th month	Gain or loss	Gain over check
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Corn stover	15.5:1	23.40	17.05	6.35	0.69	1,540	1,300	−240	0
Straw	15.0:1	23.40	17.37	6.03	1.01	1,560	1,500	−60	200
Wood fiber	16.1:1	23.40	16.90	6.50	0.54	1,460	1,405	−55	105
Bluegrass	14.1:1	23.40	18.13	5.27	1.77	1,650	1,555	−90	255
Ragweeds	13.1:1	23.40	18.73	4.67	2.37	1,790	1,815	+25	515
Tobacco stems	13.8:1	23.40	18.40	5.00	2.04	1,690	1,675	−15	375
Red clover	14.5:1	23.40	18.19	5.21	1.83	1,610	1,630	+20	330
Sweet clover	13.0:1	23.40	18.38	5.02	2.02	1,790	1,800	+10	500
Glucose	16.1:1	23.40	16.44	6.96	0.08	1,460	1,515	+55	215
Cellulose	16.1:1	23.40	17.50	5.90	1.14	1,460	1,440	−20	140
Soil check	13.3:1	19.40	16.36	3.04	....	1,460	1,300	−160	...

* Multiply by 1,000 for 2,000,000 pounds soil.

of nitrogen fixed was found with the addition of narrow-ratio materials. This would seem to indicate that nitrogen-fixing organisms are benefitted by the presence of some nitrogen in their energy material. This may be an indirect benefit, however, as other microörganisms would bring about a more rapid decomposition of such materials and this might render the nitrogen more available for nitrogen fixers.

As previously indicated one series of cultures was prepared with the view of determining the degree to which the addition of superphosphate stimulated nitrogen fixation. By comparing the amounts of nitrogen fixed (table 6) by the phosphate cultures with those of the blanks it will be noted that there is no consistent indication that phosphates have had this effect.

CONCLUSIONS

The results obtained with the Ellsworth silt loam soil employed in these experiments and under the conditions set up lead to the following conclusions:

With a given carbon-nitrogen ratio the microörganic processes taking place in the soil are similar, regardless of the organic materials added.

A ratio wider than 10 to 1 causes the loss of organic carbon from the soil.

A ratio narrower than 10 to 1 leads to the saving of organic carbon.

A wide ratio causes nitrate depression over several months whereas a narrow ratio leads to the formation of nitrates.

Wide-ratio materials may be sources of energy for nitrogen fixation, but this nitrogen will not be available to crops until losses of carbon have narrowed the carbon-nitrogen ratio to about 10:1.

Applications of phosphates do not cause appreciably greater nitrogen fixation.

Sufficient nitrogen to narrow the ratio to about 15:1 favors nitrogen fixation.

REFERENCES

(1) Albrecht, W. A. 1922 Nitrate accumulation under straw mulch. *Soil Sci.* 20: 253–265.

(2) Alway, F. J., and McDole, G. R. 1916 The loess soils of the Nebraska portion of the transition region: I. Hygroscopicity, nitrogen, and organic carbon. *Soil Sci.* 1: 192–238.

(3) Alway, F. J., and Trumbull, R. S. 1910 A contribution to our knowledge of the nitrogen problem under farming. Jour. Indus. and Engin. Chem. 2: 135–138.

(4) Alway, F. J., and Vail, C. E. 1912 The relative amounts of nitrogen, carbon, and humus in some Nebraska soils. *Nebr. Agr. Exp. Sta. 25th Ann. Rept.* 1912: 145–163.

(5) Ames, J. W., and Gaither, E. W. 1913 Composition of calcareous and non-calcareous soils. Ohio Agric. Exp. Sta. Bul. 261.

(6) Anderson, J. W. 1926 The influence of available nitrogen on the fermentation of cellulose in the soil. *Soil Sci.* 21: 115–126.

(7) Barthel, Chr., and Bengtsson, N. 1924 Action of stable manure in the decomposition of cellulose in tilled soil. *Soil Sci.* 18: 185–200.

(8) Blair, A. W., and McLean, H. C. 1917 Total nitrogen and carbon in cultivated land, and land abandoned to grass and weeds. *Soil Sci.* 4: 283–293.

(9) Blair, A. W., and Prince, A. L. 1928 The influence of heavy applications of dry organic matter on crop yields and on nitrate content of the soil. *Soil Sci.* 25: 281–289.

(10) Brown, P. E., and Allison, F. E. 1916 The influence of some common humus-forming materials of narrow and wide nitrogen-carbon ratios on bacterial activities. *Soil Sci.* 1: 49–75.

(11) Brown, P. E., and O'Neal, A. M. 1923 The color of soils in relation to organic matter content. Iowa Agr. Exp. Sta. Res. Bul. 75.

(12) Collison, R. C., and Conn, H. J. 1925 The effect of straw on plant growth. N. Y. Agr. Sta. Tech. Bul. 114.

(13) Doryland, C. J. T. 1916 The influence of energy material upon the relation of soil microörganisms to soluble plant food. N. Dak. Agr. Exp. Sta. Bul. 116.

(14) Dyer, B. 1902 Results of investigations of Rothamsted soils. U. S. Dept. Agr. Bul. 106.

(15) Hutchinson, H. B. 1919 The influence of sugar and plant residues on losses of soil nitrates. *Jour. Agr. Sci.* 9: 107.

(16) JENNY, HANS 1928 Relation of climatic factors to the amount of nitrogen in soils. *Jour. Amer. Soc. Agron.* 20: 900–913.

(17) KRISHNA, P. G. 1928 Cellulose decomposition products as sources of energy for azotobacter and B. Amylobacter. *Jour. Amer. Soc. Agron.* 20: 511–515.

(18) LYON, T. L., BIZZELL, J. H., AND WILSON, B. D. 1923 Depressive influence of certain higher plants on the accumulation of nitrates in the soil. *Jour. Amer. Soc. Agron.* 15: 457–466.

(19) MARTIN, T. L. 1925 Effect of straw on accumulation of nitrates and crop growth. *Soil Sci.* 20: 159–164.

(20) MERKLE, F. G. 1918 The decomposition of organic matter in soils. *Jour. Amer. Soc. Agron.* 10: 281–302.

(21) MURRAY, T. J. 1921 The effect of straw on biological soil processes. *Soil Sci.* 12: 233–259.

(22) READ, J. W. 1921 Practical significance of the organic carbon-nitrogen ratios in soils. *Soil Sci.* 12: 491–495.

(23) RUSSELL, E. J. 1927 Soil Conditions and Plant Growth. Longmans, Green and Co., New York.

(24) SCOTT, H. 1921 The influence of wheat straw on the accumulation of nitrates in the soil. *Jour. Amer. Soc. Agron.* 13: 233–258.

(25) SIEVERS, F. J., AND HOLTZ, H. F. 1926 The significance of nitrogen in soil organic matter relationships. Wash. Agr. Exp. Sta. Bul. 206.

(26) STARKEY, R. L. 1924 Some observations on the decomposition of organic matter in soils. *Soil Sci.* 17: 293–315.

(27) STEWART, R. 1910 Quantitative relationships of carbon, phosphorus, and nitrogen in soils. Ill. Agr. Exp. Sta. Bul. 145.

(28) THOMAS, R. P., AND HARPER, H. J. 1926 The use of oat straw in a system of soil fertility. *Soil Sci.* 21: 393–400.

(29) WAKSMAN, S. A. 1924 Influence of microörganisms upon the carbon-nitrogen ratio in soils. *Jour. Agr. Sci.* 14: 555–562.

(30) WAKSMAN, S. A. 1926 The rôle of microörganisms in the formation of "humus" in the soil. *Soil Sci.* 22: 421–436.

(31) WAKSMAN, S. A. 1927 Principles of Soil Microbiology. Williams and Wilkins, Baltimore, Md.

(32) WAKSMAN, S. A. 1929 Chemical and microbiological principles underlying the decomposition of green manures in the soil. *Jour. Amer. Soc. Agron.* 21: 1–18.

(33) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Influence of organic matter upon the development of fungi, actinomycetes, and bacteria in the soil. *Soil Sci.* 17: 373–377.

(34) WAKSMAN, S. A., AND TENNEY, F. G. 1928 Composition of natural organic materials and their decomposition in the soil: III. The influence of nature of plant upon the rapidity of its decomposition. *Soil Sci.* 26: 156–171.

(35) WAYNICK, D. D., AND SHARP, L. T. 1919 Variations in nitrogen and carbon in field soils and their relation to the accuracy of field trials. *Univ. Cal. Pub. Agr. Sci.* 4: 121–139.

(36) WHITING, A. L. 1926 Some important factors controlling the rate of nitrification of organic materials. *Jour. Amer. Soc. Agron.* 18: 854–875.

(37) WHITING, A. L., AND SCHOONOVER, W. R. 1920 The comparative rate of decomposition of green and cured clover tops in soil. *Soil Sci.* 9: 137–149.

(38) WILSON, B. D., AND WILSON, J. K. 1925 An explanation for the relative effects of timothy and clover residues in the soil on nitrate depression. N. Y. (Cornell) Agr. Exp. Sta. Mem. 95.

PHOSPHORUS ASSIMILATION BY SOIL MICROÖRGANISMS

L. G. THOMPSON, JR., F. B. SMITH AND P. E. BROWN

Iowa State College

Received for publication August 18, 1930

It is well known that microörganisms in their metabolism absorb certain soluble inorganic soil constituents. This is especially true of phosphorus, which is found in considerable amounts in microbial protoplasm. This phosphorus, on the death and decomposition of the organisms, may again be made available for plant-food.

Many microörganisms, when growing under favorable conditions, have been found to liberate carbon dioxide and bring about a change in the composition of the medium upon which they are growing. Water charged with carbon dioxide will attack many relatively insoluble soil constituents, among which is tri-calcium phosphate, from which soluble phosphates are formed. Then, too, the microörganisms, among other products of growth, may form various organic acids, which, when they come in contact with the insoluble tri-calcium phosphate, will bring the phosphorus into solution. In this connection it may be noted that there is a possibility that the phosphorus in the bodies of the dead organisms may become available through the action of autolytic enzymes without the intervention of microörganisms.

Whether these reactions will lead to an increase in the water-soluble phosphorus in the soil will depend upon whether or not the analytical processes are proceeding more rapidly than the synthetic reactions. There is also the possibility that the organic phosphorus which is soluble in pure water may be more available to the plant than is the inorganic phosphorus from which it was produced through the activity of the soil microörganisms.

In a study of cultures of 20 soil molds, Brown and Smith (1) found that *Tetracoccosporium paxianum* in solution cultures gave an increase in water-soluble phosphorus, all the others showing a decrease or a phosphate assimilation. No definite relationship between phosphate-assimilating power and change of reaction in culture solutions was shown.

The purpose of this work was to study further the assimilation of phosphorus by soil microörganisms, the utilization of various carbon compounds, acid production and its effect upon the water-soluble phosphorus of the soil.

EXPERIMENTAL

Phosphorus assimilation by molds in dextrose solution cultures

Two hundred cubic centimeters of a sterile culture medium containing 3 per cent dextrose, 0.05 per cent mono-potassium phosphate, 0.2 per cent sodium

nitrate, 0.1 per cent potassium chloride, 0.05 per cent magnesium sulfate, and 0.01 per cent ferrous sulfate in 500-cc. Erlenmeyer flasks were inoculated in triplicate with pure cultures of *Aspergillus minutus*, *Aspergillus luchuensis*, and *Aspergillus terreus*. The cultures were incubated at 25°C. for 16 days. After incubation, the reaction of the culture medium and the percentage of dextrose remaining were determined. The mycelium was removed, weighed, and the total phosphorus content determined. The acidity of the culture solution was also determined. The quinhydrone electrode was used to determine the pH, and the acidity was measured by titrating an aliquot of the medium against standard alkali. The dextrose was determined by the modified method of Shaffer and Hartmann (2). The phosphorus in the mycelium was brought

TABLE 1

Phosphorus assimilation by molds in dextrose solution cultures

ORGANISM	REACTION OF CULTURE	ACIDITY AS FORMIC ACID	WEIGHT OF MYCELIUM AFTER 2 WEEKS	PO_4 IN MYCELIUM	PO_4 ASSIMILATED	DEXTROSE IN MEDIUM
	pH	*per cent*	*gm.*	*per cent*	*mgm.*	*per cent*
Aspergillus minutus	6.76	....	1.2733	0.54	6.88	.....
Aspergillus luchuensis	2.57	9.20	1.4486	0.33	4.78	0.019
Aspergillus terreus	5.64	1.43	1.4380	0.33	4.75	0.085
Control	5.40	....	......	....	....	3.000

TABLE 2

Phosphorus assimilation by Aspergillus luchuensis in sucrose solution cultures

LENGTH OF INCUBATION	REACTION OF CULTURE	ACIDITY AS FORMIC ACID	WEIGHT OF MYCELIUM	PO_4 IN MYCELIUM	PO_4 ASSIMILATED
days	*pH*	*per cent*	*gm.*	*per cent*	*mgm.*
11	3.27	0.112	1.5800	....	.....
16	3.70	.....	1.3646	0.78	10.63
21	4.33	0.038	1.2835	....	.....
Control	5.63	0 009	......	....	.....

into solution with concentrated nitric acid and determined colorimetrically by the method of Parker and Fudge (3). The results of this experiment are given in table 1.

Phosphorus assimilation by Aspergillus luchuensis *in sucrose and dextrin solution cultures*

In the aforementioned experiment *Aspergillus luchuensis* produced a considerable acidity in the dextrose medium. This organism was selected for a further study of its growth, phosphate assimilation, and acid production using sucrose and dextrin as sources of carbon. Cultures were inoculated into a liquid medium similar to the one used in the preceding experiment, except that

3 per cent of sucrose was used instead of dextrose. Duplicate cultures were examined after 11, 16, and 21 days. In addition to the tests made in the preceding experiment, qualitative tests for various organic compounds were made. These tests showed the presence of formic, acetic, and succinic acids. No alcohols, aldehydes, nor acetones were found. The results are presented in table 2.

Qualitative tests showed an almost complete disappearance of sucrose after 21 days. No doubt some of the acids were neutralized by the sodium and potassium liberated by the utilization of nitrate and a larger phosphate assimilation. Then, too, there is the possibility that the organism decomposed the acids further. The mycelium contained 0.78 per cent phosphate at this time, 10.73 mgm. of phosphate being assimilated. The phosphorus in the mycelium was not determined after 11 and 21 days incubation. Apparently sucrose was more readily utilized than dextrose by *Aspergillus luchuensis*. The weight of mycelium produced by *Aspergillus luchuensis* in sucrose solution decreased with increasing incubation period. This was probably due to a solution of the mycelium, since the energy material had been quickly utilized and the organism could make no further growth.

TABLE 3

Phosphorus assimilation by Aspergillus luchuensis in dextrin solution cultures

LENGTH OF INCUBATION	REACTION OF CULTURE	ACIDITY AS FORMIC ACID	WEIGHT OF MYCELIUM	PO_4 IN MYCELIUM	PO_4 ASSIMILATED
days	*pH*	*per cent*	*gm.*	*per cent*	*mgm.*
22	4.85	0.046	1.0685	Trace	......
32	....	0.055	1.1270	0.03	0.3381
38	4.92	0.046	1.0560	0.09	0.9504
Control	5.43	.....	.....	....	......

Growth of this organism was very slow in a dextrin solution medium similar to the sucrose medium, except that dextrin was used to supply carbon and a part of the phosphate was supplied as di-ammonium phosphate and a part as mono-potassium phosphate (table 3). The acidity of the medium was increased but slightly and the percentage of titrable acidity was low, probably because of the liberation of some ammonia from the ammonium phosphate. The amount of mycelium produced in 22 days in dextrin solution medium was but slightly less than the amount produced in dextrose or sucrose in 16 days but the phosphorus content of the mycelium was low and only 0.9504 mgm. of phosphate were assimilated after 38 days.

Phosphorus assimilation by Azotobacter chroococcum *in soil cultures*

Another experiment was planned to determine the effect of a pure culture of *Azotobacter chroococcum* upon the water-soluble phosphorus of the soil, and also upon chemically pure tri-calcium phosphate when added to the soil. The

effect of the native soil flora upon the solubility of tri-calcium phosphate was also determined.

One hundred grams of Carrington loam were placed in each of 54 500-cc. Erlenmeyer flasks. There were two series as follows:

Flask No.	
	Series A sterile
1, 2, 3	Check
4, 5, 6	Inoculated with *Az. chroococcum*
7, 8, 9	1 gm. of tri-calcium phosphate added
10, 11, 12	1 gm. of tri-calcium phosphate added and inoculated with *Az. chroococcum*
	Series B not sterile
13, 14, 15	Check
16, 17, 18	1 gm. of tri-calcium phosphate added

One-half gram of dextrose was added to each flask. The moisture content of the soil was adjusted to 50 per cent of the saturation capacity by the addition of sterile, distilled water. Three sets of 18 flasks each were made up; one set was incubated for 21 days, the second set for 45 days, and the third set for 90 days. At the end of each incubation period the water-soluble phosphorus was determined by the colorimetric method of Parker and Fudge (3).

TABLE 4

Phosphorus assimilation in Carrington loam by Azotobacter chroococcum

TREATMENT	WATER-SOLUBLE PHOSPHORUS					
	After 21 days	Assimilated in 21 days	After 45 days	Assimilated in 45 days	After 90 days	Assimilated in 90 days
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Sterile check	3.8	...	3.6	...	3.8	.....
Sterilized and inoculated with *A. chroococcum*	1.4	2.4	0.6	3.0	4.6	−0.8
Sterilized and 1 gm. $Ca_3(PO_4)_2$ added	31.3	...	31.0	...	28.6	.....
Sterilized, 1 gm. $Ca_3(PO_4)_2$ added and inoculated with *A. chroococcum*	28.0	3.3	25.6	5.4	34.2	−5.6
Unsterile check	Trace	...	0.2	...	0.8	.....
Unsterilized and 1 gm. $Ca_3(PO_4)_2$ added	43 6	...	40.6	...	61.2	.....

Table 4 gives the amount of water-soluble phosphorus in parts per million for the various treatments and the different incubation periods, and the phosphate assimilated. For the first 21 days there was a decrease of 2.4 milligrams and for 45 days, 3.0 milligrams in water-soluble phosphorus in the sterile soils inoculated with *Azotobacter chroococcum* as compared to the sterile check soils. This was found to be true in both the untreated soils and in the soils treated with tri-calcium phosphate, the decrease being larger in the phosphate-treated soils. This indicates that under the conditions of the experiment, *Azotobacter chroococcum* assimilated more phosphorus than was made available by their

analytic processes. During the next 45 days there was an increase in water-soluble phosphorus in the inoculated soils. This was probably due to the death and decomposition of the Azotobacter cells through the action of autolytic enzymes, thus liberating water-soluble phosphorus.

When the sterile check soil to which tri-calcium phosphate was added, is compared with the unsterile soil receiving the same treatment, it is apparent that the natural soil flora increased the amount of water-soluble phosphorus for the first 21 days. This indicates that the soluble phosphorus formed by the analytic reactions exceeded the phosphorus absorbed by the synthetic reactions. During the next 24 days there was a slight decrease in water-soluble phosphorus, which was probably due to the more rapid growth of certain species or the dying out of certain other species thus changing the ratio between the analytic and the synthetic reactions. For the next 45 days there was a large increase in water-soluble phosphorus in the unsterile soil, which may be due partly to the death and decomposition of the phosphate-assimilating bacteria and partly to a shift in species relationships in the soil.

In the case of the unsterile soil, to which no tri-calcium phosphate was added the quantity of water-soluble phosphorus increased during the course of the experiment, showing that the natural soil flora of this soil at least, assimilated less phosphorus than was made available by their analytic processes.

SUMMARY AND CONCLUSIONS

Aspergillus luchuensis increased the hydrogen-ion concentration of liquid culture media considerably in 11 days to two weeks but the reaction became less acid as the incubation period was increased. *Aspergillus minutus* decreased the hydrogen-ion concentration in dextrose solution cultures after two weeks. This organism did not produce as much mycelium as *Aspergillus luchuensis* but it contained a larger percentage of phosphorus and assimilated more phosphate. If the assimilation of phosphorus is accompanied by a decrease in the hydrogen-ion concentration of the culture medium, *Aspergillus minutus* may have produced more acidity than *Aspergillus luchuensis*, the acidity being neutralized earlier in the incubation period. The phosphorus assimilation by *Aspergillus luchuensis* was small in dextrin and sucrose solution cultures. *Azotobacter chroococcum* assimilated more phosphorus for the first 45 days than was made available, but for the second period of 45 days more phosphorus was made available than was utilized. The addition of insoluble phosphate increased the amount of phosphorus assimilated during the first 45 days of the experiment and also increased the amount of water-soluble phosphorus during the second 45 days. The native soil flora of Carrington loam was more effective in dissolving tri-calcium phosphate than was *Azotobacter chroococcum*. Different species of soil microörganisms probably affect the water-soluble phosphorus differently, for some species may have a greater dissolving action than assimilating power, whereas with other species the reverse may be the case.

REFERENCES

(1) Brown, P. E., and Smith, F. B. 1930 Phosphorus assimilation by certain soil molds. *Iowa State Col. Jour. Sci.* 3: 325.

(2) Fred, E. B., and Waksman, S. A. 1928 Laboratory Manual of General Microbiology, 72. McGraw-Hill Book Company, Inc., New York.

(3) Parker, F. W., and Fudge, J. F. 1927 Soil phosphorus studies: I. The colorimetric determination of phosphorus in soil extracts and soil solutions. *Soil Sci.* 24: 109.

CHEMICAL METHODS FOR ESTIMATING THE AVAILABILITY OF SOIL PHOSPHATE

P. L. HIBBARD

University of California[1]

Received for publication August 13, 1930

The purpose of this paper is to record the main points of interest developed by the writer during the past three years of an investigation of chemical methods for determining the availability to plants of soil phosphate. Since the days of Liebig this has been a live question, and it still lacks a satisfactory answer.

Available soil phosphate is, for the present purpose, defined as "that part of the soil phosphate which may be absorbed or used by ordinary crop plants in the production of plant substances." Also it is assumed that different species of plants may have quite unlike power to obtain an adequate supply of phosphate from a given soil which is deficient in available phosphate.

In the endeavor to find a somewhat satisfactory procedure, several methods proposed by others have been examined (the results are reported at the end of this paper), much experimental work has been done, and some new methods have been worked out. It is thought that some of the new methods permit a closer approach to an adequate solution of the problem than any previously published chemical methods.

INTRODUCTION

A satisfactory estimation of the availability of soil phosphate by chemical methods may be difficult or impossible for several reasons. Some are here mentioned.

Phosphorus occurs in the soil in several forms of combination, both mineral and organic. This paper is concerned with mineral phosphate only. Apatite, $Ca_3(PO_4)_2CaF_2$; chlorapatite, $Ca_3(PO_4)_2CaCl_2$; hydroxyapatite, $(Ca_3(PO_4)_2)_3Ca(OH)_2$; wagnerite, magnesium phosphate; and wavellite, aluminum phosphate, are soil minerals. According to Russell (21), hydroxyapatite is probably the chief source of soil phosphate. These minerals are relatively insoluble in water. Since most mineral phosphates are very insoluble in water, there is but little PO_4 dissolved in the soil solution at any one time. The amount of PO_4 in solution is increased by CO_2 or other acids, provided they do not at the same time increase too much the concentration of those cations which tend so repress the dissolving of phosphate.

[1] Division of Plant Nutrition

Some students believe that plants have power to absorb certain nutrients from the solid state, without first having to be dissolved in the soil solution. This is said to be true of phosphates. Also plants have the power of selection so that they can take up PO_4 without at the same time absorbing the cations combined with it.

Another difficulty in estimating the availability to plants of soil phosphate by chemical means is found in the fixation of PO_4 by the soil so that it becomes insoluble in the soil solution and largely unavailable to plants. The determination of whether fixation is a purely chemical phenomenon or is more or less dependent on surface action and colloidal effects has not been made a part of this study. It seems probable that the availability of fixed phosphate depends much on the form in which it is fixed, particularly as to whether it is combined with Fe, Al, or Ca, but chemical agents do not well separate these different phosphates.

Even though the supply of available phosphate is adequate, plants may fail on account of the lack of some other nutrient or perhaps from the presence of some unknown or unsuspected injurious or toxic substance or condition, but such adversities to plants will have little effect on the solubility of soil phosphate in chemical reagents. Since chemical methods of estimating the availability of phosphate depend on first getting it into solution, and since chemical solubility may be very little dependent on several of the aforementioned factors, it is evident that chemical methods can not well be expected to imitate the action of plants in estimating the availability to plants of soil phosphate.

After plants have grown upon a soil which has been treated with phosphate, it seems unreasonable to expect that any chemical test applied to the cropped soil will give a proper representation of the soil as the plant had to deal with it at the start immediately after the phosphate was added, particularly if the phosphate was mostly water-soluble at first.

EXPERIMENTAL

To enable the reader to understand better the experimental work recorded in the following, there is presented on page 439 a tabular statement giving the laboratory number, name, response to phosphate fertilizers, and general fertility of the soils most used in this work.

The methods used in obtaining soil extracts for this study are of two kinds: equilibrium methods, in which the soil is shaken with a volume of solution for some time, then the filtered solution is analysed; and percolation methods, by which the soil is percolated with a solvent to more or less complete removal of PO_4 or other ions, then the percolate is examined. The numerical results obtained by these two procedures are usually unlike and not properly comparable. It is not intended to convey the idea that complete equilibrium is attained in the first case, but only a near approach to it; or that complete extraction, only approximate, is obtained by percolation. It is thought that in either case the process was carried far enough to show the value of the procedure.

Character of the soils studied

NUMBER	NAME	RESPONSE TO PO_4 FERTILIZER	GENERAL FERTILITY
1C	Yolo silty clay loam	Little	Very good
30	Fresno fine sandy loam	None	Very good
36	Farwell sandy loam	Large	Low
37	Nord sandy loam	Large	Low
38	Vina silty clay loam	Little	Good
40	Altamont-Olympic loam, wash	None	Very high
45	Aledo, Ill., clay loam and rock phosphate	....	Good
46	Aledo, Ill. Same untreated	....	Poor
53	Delhi sand	Some	Fair
59	Aiken clay	Very great	Medium
64	Vina silt loam	Great	Poor
65	Yolo loam	Little	Very high
66	Aiken clay	Great	Medium
68	Tejunga fine sand	Little	Good
69	Sites clay loam	Great	Medium
78	Fine sandy loam	Great	Poor
80	Hanford fine sandy loam	Great	Poor

Equilibrium methods for giving an idea of the available PO_4 in a soil are subject to the general criticism that the effects produced in this way are very unlike those produced by a plant drawing nutrients from the soil in a selective manner. In an equilibrium extraction, some of the products of the action of weak solvents may tend to repress solubility of PO_4, e.g. Ca and Mg brought into solution by weak or dilute acids. This has been pointed out by Teakle (23) and others.

It seems irrational to apply such concentrated acids as have commonly been used, such as strong HCl; 0.2 N HNO_3, pH 0.7; or 1 per cent citric, pH 2.1, since plants have no such strong solvent agents at their disposal.

Perhaps the best reason for using equilibrium methods is that they may supply useful indications with the least expense of time and labor. A fair idea of the power of a soil to supply PO_4 may be quickly had in this way, but at the same time the result may give an erroneous notion of the availability to plants of the PO_4 thus extracted.

Equilibrium methods

Ratio of soil to solution 1:5. Usually 40 gm. of soil was placed in a 400-cc. bottle with 200 cc. of solution and agitated in an end over end shaker for three hours or more. The mixture was filtered on Whatman No. 12, 24 cm. folded filters, and after nearly all the liquid had run through, the solution was analysed. PO_4 was determined by the molybdenum blue method about as described by Parker and Fudge (19), pH by color comparison, Ca by turbidity after adding $(NH_4)_2O_x$, Fe by color comparison after adding $K_4Fe(CN)_6$ and HCl.

A single extraction of any soil by any solvent which changes the pH materially gives only a very incomplete picture of the character of the soil in regard to its power to supply phosphate under changing conditions. But by using three or four different concentrations of a dilute acid, much more is learned. In this, it is important to note the pH of the solution removed from the soil. This presumes that only dilute acids are used, so that the pH of the extract never is brought below 2.

In this mannei, more than 50 different soils have been examined. A few typical results are presented in table 1, which gives the figures obtained with citric, oxalic, and hydrochloric acids of 0.005, 0.025, 0.050, and 0.100 normalities. This shows the great differences between different soils and also the different results given by the different acids of the same concentration on any one soil. These figures indicate something of the buffer power of the soil, or its power to neutralize acid, how much acid is needed to reduce the pH of the soil to some lower point, and how much PO_4 may be brought into solution at that pH. It is apparent that the amount of PO_4 dissolved is closely related to the pH of the solution when simple acids such as HCl or HNO_3, and probably also CO_2, are used. Citric and oxalic acids form soluble complexes with Fe^{+++}, and other cations, so have greater solvent effect than their concentration of hydrogen ion should give of itself. Oxalic acid is peculiar in that it removes Ca from solution at pH above 3 to 4, thus permitting more PO_4 to remain in solution than would HCl, H_2SO_4, or HNO_3. Also, citric and oxalic acids are not appropriate because they must be removed from the solution before it is tested for PO_4 content by the molybdenum blue method. Carbon dioxide in equilibrium at ordinary atmospheric pressure has too small a concentration of hydrogen ion to be an active solvent. But when used continuously as in percolation with water saturated with CO_2, it has great solvent effect since the products of reaction are continually removed, thus allowing the reaction to proceed to completion in one direction. This largely accounts for the great effects on the soil produced by rain water.

Buffer power of the soil in relation to the solution of soil phosphates by dilute acids. The figures appearing in table 1 show how different is the effect of a dilute acid on different soils. It seems evident that instead of using the same arbitrary concentration of acid on all soils, an amount of acid should be used such that the pH of the extract will be the same for all soils. If this is accomplished the results should be properly comparable. The figures given in table 1 may be plotted so that by interpolation or extrapolation the PO_4 soluble at any definite pH, such as 4, may be inferred. Figure 1 gives such graphs for a few soils, and table 2 gives PO_4 at pH 4 for several soils. These figures seem to give a much better idea of the phosphate-supplying power of the soil than do those found by the use of the same amount of acid for all soils, in which case the pH of the extracts often differs greatly.

There are two objections to this method. Unless two points somewhere near pH 4 happen to be hit in the determinations, the figure inferred for pH 4

may be wide of the truth, and this method requires the preparation and analysis of three or four solutions. To avoid these objections, it was sought to make the extraction by the use of such an amount of acid that the extract would have a pH of approximately 4. This was found difficult to accomplish in a simple practical way because when a soil is mixed with a dilute, highly ionized acid, the pH does not remain at one point for more than a few minutes, and because it is necessary to make some preliminary experiments to indicate about how much acid will be needed to bring the pH of the soil to any definite point.

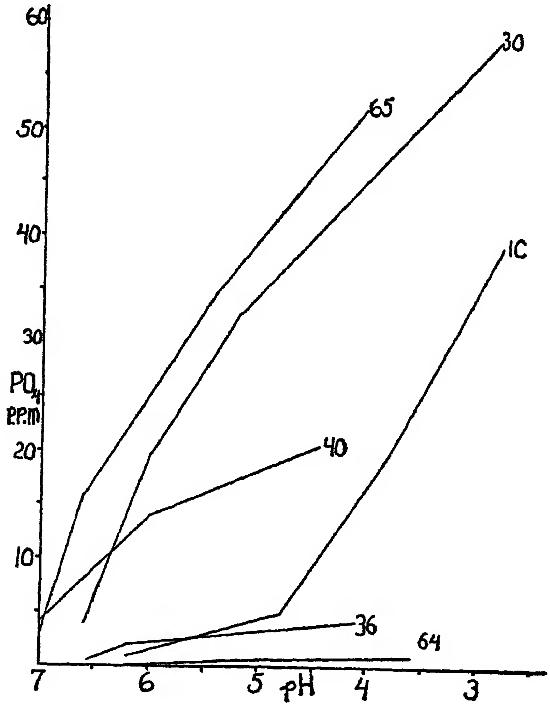


FIG. 1. RELATION OF PO_4 IN DILUTE ACID EXTRACT TO pH OF THE EXTRACT

The figures given in table 3 show how rapidly the solution changes after acid has been added and how greatly soils differ in this respect when the highly ionized, slightly buffered HCl is used. Addition of KCl, suggested by Truog (24), to the acid did not give enough buffer effect to be of any advantage. When citric acid was used, its buffer power made the change in content of PO_4 of the solution much slower, but it is undesirable for reasons stated on page 440. Acetic acid is little ionized, highly buffered, and otherwise an appropriate solvent. Although the PO_4 content of the extract made with this acid is less constant in some cases than when citric acid is used, it seems better adapted

for estimating available PO_4 than any of the other common acids. Oxalic acid, suggested by Vanstone (25), is even less appropriate than citric acid. Some of the results obtained with acetic acid are given in table 4.

TABLE 1

PO_4 and pH in 1:5 equilibrium extracts of soils with citric, oxalic, and hydrochloric acids of four different concentrations

SOIL NUMBER	ACID	0.005 *N* ACID		0.025 *N* ACID		0.05 *N* ACID		0.1 *N* ACID	
		PO_4 *p.p.m.*	*pH*	PO_4 *p.p.m.*	*pH*	PO_4 *p.p.m.*	*pH*	PO_4 *p.p.m.*	*pH*
1C	Citric	5	5.2	16	4.2	27	3.6	44	3.0
	Oxalic	10	5.2	27	4.4	50	3.3	66	2.4
	HCl	5	4.8	20	3.8	40	2.8	40	1.5
30	Citric	24	4.2	33	3.3	40	2.9	57	2.6
	Oxalic	40	3.9	66	2.4	80	1.8	80	1.4
	HCl	40	4.8	80	2.8	80	1.5	80	1.5
65	Citric	40	5.2	50	4.2	66	3.6	66	3.0
	Oxalic	40	5.3	44	4.0	56	3.0	80	2.0
	HCl	40	5.2	56	3.0	80	2.0	80	1.5
38	Citric	....	...	10.0	4.4	13	3.7	20	3.0
	Oxalic	....	...	8.0	4.8	20	3.6	50	2.4
	HCl	....	...	3.2	4.0	6.6	2.4	10	1.4
64	Citric	....	...	8.8	4.5	13	3.6	22	3.0
	Oxalic	....	...	8.0	5.0	20	3.6	66	2.4
	HCl	....	...	7.2	3.6	10	2.2	13	1.5
35	Citric	....	...	5.6	3.8	9.0	3.2	11.0	2.8
	Oxalic	....	...	2.0	4.4	5.0	3.4	33.0	2.4
	HCl	....	...	0.8	3.2	1.2	2.0	2.4	1.4
59	Citric	0.2	5.2	0.4	3.9	0.3	3.2	0.3	2.5
	Oxalic	0.2	5.0	1.0	4.4	0.4	3.4	1.6	2.5
	HCl	0.1	4.2	0.1	3.0	0.1	2.0	0.1	1.0
37	Citric	1.0	7.5	1.2	7.3	1.6	7.0	10.0	4.4
	Oxalic	1.0	7.5	1.2	7.3	3.2	6.8	11.4	4.5
	HCl	0.6	7.5	1.2	7.0	3.2	5.6	11.4	4.0
36	Citric	....	...	13	4.4	20	3.6	26	3.0
	Oxalic	....	...	16	4.8	50	3.6	80	2.4
	HCl	....	...	8	4.0	20	2.2	40	1.8

Probably a much larger proportion of solvent to soil would make it easier to hold the pH of the extract to some definite point. In table 5 are shown some of the results obtained with 2 gm. of soil to 200 cc. of solution. Although the

concentration of PO_4 in the extract is much higher in the 1:5 extracts, the amount dissolved from the soils is much greater in the 1:100 extracts. The greater proportion of water dissolves much more PO_4 from the soil. In the more dilute extracts the relative supplying power of the different soils is quite different from that found by the 1:5 extraction. But the latter represents much better the actual phosphate-supplying power of the soils as indicated by growth of plants on them.

This brings out the point that in most soils only a small proportion of the phosphate present dissolves in weak solvents at small dilutions. It seems reasonable to think that the nearer the ratio of soil to solution is to the ratio existing in a soil where plants are growing, the nearer will the results for PO_4

TABLE 2

PO_4 in 1:5 equilibrium extract of soils with dilute HCl, at pH 4 by interpolation

FERTILITY OF SOIL	SOIL NUMBER	PO_4 AT pH 4
		p.p.m.
Very fertile	1C	17
	30	44
	40	23
	65	52
Good	36	4.0
	37	11.0
	38	1.5
	68	14.0
Aledo, Ill., and rock phosphate	45	13.0
Aledo, Ill., not fertilized	46	1.4
Very poor	64	1.0
Exceedingly poor	59	0 1
	66	0.1
	69	0.4
	76	0.2

represent the condition with which the plant has to deal. The work of others, as well as some done during this study, shows that the concentration of PO_4 in water extracts of some soils is almost the same regardless of the ratio of soil to solvent until large dilutions are reached. Results obtained in this study are shown in table 6.

The same thing is shown in table 7, giving the results from extracting several soils with dilute acetic acid with four different ratios of soil to solvent. In this experiment, soils 64, 38, 89B, and 91A gave somewhat the same concentration of PO_4 in the solution whether the ratio of soil to solution was 1/5 or 1/40.

Soils 1C, 30, 36, 68, 75, and 80 show increasing amounts of PO_4 extracted from the soil as the ratio of solvent to soil is increased, yet the increase in PO_4

is not equal to the increase in dilution. In table 5 are some figures found by a dilution of 1/100, and the other extreme is shown in table 8, with a ratio of 2/1. Pot cultures show that the PO_4-supplying power of the soil is much better represented by the figures given in table 7. The chief reason for using a 1:5 ratio instead of 2:1 of soil and solvent, is that it is much more convenient, and still the dilution is not so great that the results are not applicable.

TABLE 3

Change in pH and PO_4 with change in time after acid has been added

120 gm. soil shaken with 600 cc. of solution

SOIL NUMBER	0.1 *N* HCl ADDED	TIME FOR REACTION	SOLUTION AFTER FILTERING	
			pH	PO_4
	cc.			*p.p.m.*
1C	300	10 minutes	2.05	50
		1 hour	2.21	50
		5 hours	2.62	29
		24 hours	3.13	10
		72 hours	3.38	4
30	120	10 minutes	2.05	40
		1 hour	2.17	66
		5 hours	2.31	100
		24 hours	2.53	111
		72 hours	2.70	117
35	200	10 minutes	2.07	0.88
		1 hour	2.57	0.58
		5 hours	3.19	0.40
		24 hours	3.54	0.28
		72 hours	3.54	0.20
36	300	10 minutes	2.09	25.0
		1 hour	2.39	25.0
		5 hours	2.66	15.0
		24 hours	3.00	5.0
		72 hours	3.21	2.4
38	170	10 minutes	2.79	4.8
		1 hour	3.17	2.8
		5 hours	3.73	1.7
		24 hours	4.09	0.8
		72 hours	4.05	0.7
64	270	10 minutes	2.01	11.4
		1 hour	2.31	8.8
		5 hours	2.55	4.8
		24 hours	2.87	1.6
		72 hours	3.12	1.1

TABLE 4

Change in pH and PO_4 with time after acid has been added

80 gm. soil shaken with 400 cc. solution

SOIL NUMBER	TIME FOR REACTION	0.1 *N* CITRIC ACID	pH	PO_4 IN SOLUTION	0.1 *N* ACETIC ACID	pH	PO_4 IN SOLUTION
		cc.		*p.p.m.*	*cc.*		*p.p.m.*
1C	5 minutes	70	4.13	8.0	250	....	.....
	75 minutes		4.29	11.4		4.04	12
	5 hours		4.46	11.4		4.04	10
	16 hours		4.58	11.4		4.09	8
	48 hours		4.90	11.4		....	.....
36	5 minutes	70	4.18	3.4	270	....	.....
	75 minutes		4.32	6.6		4.01	6.4
	5 hours		4.40	6.6		3.99	4.4
	16 hours		4.51	6.6		4.05	2.8
	48 hours		4.70	6.6		....	.....
38	5 minutes	70	4.13	1.4	220	....	.....
	75 minutes		4.32	2.3		4.05	1.44
	5 hours		4.51	2.4		4.09	1.24
	16 hours		4 74	2.4		4.13	1.14
	48 hours		5.17	1.8		....	.....
30	1 hour		....	....	100	3.97	19
	5 hours		....	....		3.97	22
	24 hours		....	....		4.05	25
64	5 minutes		....	....	220	3.96	3.2
	1 hour		....	....		4.05	2.0
	5 hours		....	....		4.03	1.2

TABLE 5

PO_4 extracted from soils by dilute acetic acid at approximately pH 4

2 gm. soil to 200 cc. solution shaken 1 hour

SOIL	0.1 *N* H Ac IN 200 CC.	pH	PO_4 IN SOLUTION
	cc.		*p.p.m.*
1C	10	4.1	4.0
30	5	4.1	2.4
36	10	4.1	2.4
37	20	4.2	2.9
38	10	4.0	1.2
89B	5	4.0	1.2

The important points in the preceding discussion may now be summed up to show what facts and conditions should be considered in devising a method for

TABLE 6

PO_4 in equilibrium extracts of soils with several different proportions of ordinary distilled water

SOIL NUMBER	RATIO OF SOIL TO WATER									
	1/1	1/2	1/4	1/10	1/20	1/50	1/100	1/500	1/1,000	1/2,500
	PO_4 p.p.m. in solution									
1C	0.20	....	0.20	....	0.20	....	0.16	0.20	0.12	0.02
30	4.40	....	3.60	....	2.00	....	0.70	0.16	0.02	0
36	....	0.50	....	0.40	....	0.24	0.20	0.16	0.08	0
37	....	0.50	....	0.60	....	0.28	0.12	0.10	0.04	0
38	0.32	....	0.40	....	0.12	....	0.20	0.12	....	0.06
35	....	....	0.04	....	0.04	....	0.06	0.10	....	....

TABLE 7

PO_4 in acetic acid extracts of soils with various weights of soil to 200 cc. of solvent mixture, shaken one hour before filtering

SOIL NUMBER	SOIL	0.1 N H Ac	pH	PO_4 IN SOLUTION	SOIL NUMBER	SOIL	0.1 N H Ac	pH	PO_4 IN SOLUTION
	gm.	*cc.*		*p.p.m.*		*gm.*	*cc.*		*p.p.m.*
1C	5	30	3.97	6.8	68	5	25	3.75	3.0
	10	46	3.97	8.8		10	35	3.71	4.4
	20	77	3.95	11.4		20	55	3.67	5.4
	40	140	3.95	13.2		40	95	3.65	7.2
30	5	21	3.85	6.0	75	5	30	3.90	12.0
	10	28	3.83	9.0		10	45	3.96	16.0
	20	40	3.85	14.5		20	75	3.92	27.0
	40	65	3.85	25.0		40	140	3.90	40.0
64	5	29	3.92	3.1	89B	5	20	3.85	1.4
	10	43	3.94	2.7		10	25	3.73	1.8
	20	70	3.92	2.2		20	30	3.97	1.2
	40	125	3.90	2.2		40	40	4.01	1.1
38	5	35	3.94	2.0	91A	5	20	3.99	1.4
	10	50	3.96	2.0		10	27	4.07	1.2
	20	85	3.92	2.0		20	42	4.01	1.0
	40	150	3.92	2.0		40	70	3.97	1.0
36	5	30	3.96	3.6	92A	5	23	4.01	4.4
	10	46	3.96	4.8		10	30	4.05	3.6
	20	80	3.90	6.2		20	45	4.03	2.7
	40	145	3.92	8.0		40	73	4.05	1.5
80	5	30	4.16	6.0		..	..	....	....
	10	45	4.23	9.0		..	..	....	....
	20	75	4.25	14.5		..	..	....	....
	40	140	4.29	20.0		..	..	....	....

determining availability of soil phosphates by equilibrium extracts and to give results numerically properly comparable:

Soils differ in power to neutralize acids, "buffer power." Acids bring into solution cations which tend to remove PO_4 from solution between pH 3 and 8.

Chemical reagents have not the selective power of plants, and plants have no strong solvent powers similar to strong acids.

The best kind and strength of acid for estimating the availability of soil phosphates are not known, but it should not be a strong, highly ionized, slightly buffered acid, since such will not hold the pH or PO_4 constant for more than a few minutes.

A highly buffered acid makes it much easier to hold a constant pH and approximately constant PO_4, though in any case PO_4 in solution changes even if pH is held constant, but citric and oxalic acids are not appropriate because they form soluble complexes with Ca, Mg, Al, and Fe, thus preventing them from having their normal effect on solubility of PO_4 at the corresponding pH.

TABLE 8

Equilibrium extracts

200 gm. soil with 100 cc. dilute acid

SOIL NUMBER	0.1 *N* HCl USED	pH OF EXTRACT	PO_4 IN EXTRACT	TIME ALLOWED FOR REACTION
	cc.		*p.p.m.*	*hours*
1C	60	4.0	1.6	15
30	30	4.0	40.0	4
35	50	3.7	0.2	4
36	100	4.4	1.3	15
37	60*	5.2	2.6	15
38	55	4.8	0.4	15
64	75	4.2	0.3	15
76	30	3.4	0.8	4
79	50	3.8	0.2	4

* *N* HCl.

Acetic acid has not these defects, is well buffered, and is convenient.

To be properly comparable, all extracts should have approximately the same pH, say 4, and all other conditions of making the extracts, such as time, volume of solution, and method of shaking, should be similar.

Time required to reach equilibrium is not known but if 24 hours be taken arbitrarily, it will be sufficient for most soils and the results will be comparable for all.

The amount of acid needed for any particular soil must be found by experiment.

Most previous investigators have not controlled these conditions adequately in order to obtain properly comparable results.

To avoid these difficulties as much as possible, the following described procedure is proposed as being practical, easily executed, not very time-consuming or expensive, and as giving results properly comparable, as well as may be expected of any empirical procedure.

Availability test for soil phosphate. A preliminary test is made to learn ap-

proximately how much acid is needed to bring the soil to pH 4. Place 10 gm. of soil in a 100-cc. bottle with 10 to 20 cc. of water. Close the bottle with a soft rubber stopper and shake vigorously a few seconds. Remove the stopper and press the end against a white porcelain plate, making a mud spot. Place a drop of suitable indicator on the mud spot. This gives a good indication of the pH of the soil. Now add a little 0.1 N HAc to the mud in the bottle, shake again, make another mud spot, and determine the pH. In this way, continue until a pH of 4 is obtained. This requires only a few minutes. Next calculate how much acid will be needed to bring 40 gm. of soil to pH 4. To each of three 300-cc. bottles add 40 gm. of soil and 200 cc. of dilute acid. One bottle should have about half as much acid as is calculated to bring the soil to pH 4, one bottle should contain the estimated amount and the other considerably more than that amount of acid, but the total liquid should be the same in all three.

Shake the bottles in an end over end shaking machine for 5 hours, let stand over night, then shake again for 3 to 4 hours. Shaking by hand is much less effective, and unlikely to give comparable results. After the shaking is finished, pour the contents of the bottles upon fluted filters. The filters should be previously moistened with dilute HCl and washed free of acid by water, so that they will not change the pH or PO_4 content of the extract. Some of the first of the filtrate is rejected. After the remainder is collected, the pH is determined and the PO_4 is estimated by the molybdenum blue method. Parker's (19) procedure is followed.

In this way, three values of pH and PO_4 for each soil are found. These figures may be plotted; then from the graph, the PO_4 of the soil at exactly pH 4 may be read off.

This test gives information in regard to the buffer power of the soil, how it will be affected by acid, and how much PO_4 will be dissolved at pH 4 or some other convenient point.

Rate of solubility curve. By using several different ratios of soil to solvent, Vanstone (25) derives from the results a curve indicating the relative availability of soil phosphate. In his examples, the chemical tests seem to agree fairly well with field results. Similar results are given by Simon (22). In applying this method to several of the soils used in this work the results do not show the same availability of soil PO_4 as is shown by pot and plot tests with plants. The laboratory results are set out in table 6. Just as in other equilibrium studies, the figures obtained for most soils show fairly well the relative availability of the soil PO_4. But some marked exceptions with all these methods greatly reduce the value of the results as a means of estimating the fertility of an unknown soil. In this respect, the Vanstone method seems no better than most other equilibrium methods. In all the modifications of this method here tried, the results obtained for soils 38, 64, and 80 do not agree with results of pot cultures. In the latter, soil 38 has generally shown a good availability of PO_4 to tomatoes, alfalfa, and barley, whereas soils 64 and 80 fail to give satisfactory

crops without added PO_4, yet the chemical equilibrium tests indicate that these soils contain more avilable PO_4 than soil 38.

Percolation methods

The percolation method of extracting PO_4 from a soil is more like the action of plants than the equilibrium method, since it removes from the soil the products of the action of the solvent, and thus permits the reaction to go toward completion in one direction. In beginning this study, it was hoped that percolation methods would give some measure of the power of a soil to renew PO_4 in the soil solution after the easily soluble PO_4 had been removed. J. W. Tidmore and L. Meyer, working independently in this laboratory, at about the same time sought to attain the same end by different methods (see p. 461).

The methods, described in the following, have been in use here for three years, have been applied to hundreds of different soils, and have given results in most cases according fairly well with the character of the soil as shown by growth of plants in pots or in the field.

Two concentrations of acid, 0.001 N and 0.05 N, have been used. The procedure is essentially the same with both, though the apparatus used with the more dilute acid is capable of much more precise control so that the results are more easily reproducible.

The details of procedure are about as follows, so far as the soil is concerned.

Twenty grams of the soil is placed in a filter tube 48 mm. in diameter. The soil rests on a bed of filter paper pulp 5 to 8 mm. thick, supported on a filter plate. If the soil is fine in texture, some acid-washed white sand is mixed with it to aid in percolation. This sand is quite fine, nearly pure quartz. It has a slight fixing power so that a small amount of PO_4 is retained by the sand when a dilute solution is passed through it. However, it was found that after 1 liter of 0.001 N HCl had been passed through it, practically the whole 0.2 mgm. of PO_4 added to the sand was recovered. So there is no fear that the sand will retain any of the PO_4 extracted from the soil.

When the filter tube with the soil and with added sand, if necessary, is set up it is connected with an elevated reservoir of the dilute acid by a pressure tube about 1.6 m. long, and the acid is allowed to percolate through the soil at the rate of about 1/3 liter an hour continuously for about 20 hours until very little PO_4 is found in the percolate or until a definite volume of percolate has been collected. Originally the percolation was continued until nearly complete extraction of the PO_4 soluble in that reagent was obtained. Lately the procedure has been standardized so that 7 liters of percolate are taken for each soil.

Percolation with 0.001 N *HCl.* With this procedure, the percolate is collected in a train of liter bottles in such manner that the contents of the seven bottles represent successive stages in the extraction. The percolates are analyzed separately so that the rate of extraction of PO_4, and the Ca and the pH of each may be determined. When the apparatus is set up and percolation started, it proceeds automatically without attention except to see that the supply reser-

TABLE 9

PO_4 extracted from soils by percolation with 0.001 N and 0.05 N HCl

SOIL NUMBER	ACID NORMALITY	PO_4 IN SUCCESSIVE 1-LITER PORTIONS OF PERCOLATE							TOTAL EXTRACTED	TOTAL IN SOIL	AVAILABLE
		1	2	3	4	5	6	7			
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*mgm.*	*p.p.m.*	*per cent*
1C	0.001	1.50	2.65	2.60	2.65	2.50	2.00	1.65	15.75	787	82
	0.05	17.00	1.10	0.5	0.30	0.30	0.20	0.10	19.30	965	
75	0.001	3.60	4.40	4.40	3.20	1.60	0.80	0.52	18.92	946	78
	0.05	18.00	2.70	1.00	0.54	0.54	0.54	0.40	23.76	1,188	
30	0.001	5.00	4.40	2.70	2.00	1.10	1.00	0.90	17.10	855	76
	0.05	20.00	1.00	0.50	0.30	0.30	0.10	0.10	22.30	1,115	
53	0.001	2.10	1.80	1.50	1.20	0.80	0.60	0.5	8.35	417	91
	0.05	6.40	1.60	0.50	0.22	0.20	0.14	0.10	9.16	458	
68	0.001	5.00	6.20	4.40	2.80	2.00	1.40	1.0	22.80	1,140	92
	0.05	22.00	2.00	0.40	0.20	0.10	0.10	0.0	24.80	1,240	
78	0.001	0.14	0.16	0.12	0.08	0.06	0.04	0.04	0.64	32	71
	0.05	0.40	0.16	0.10	0.08	0.07	0.06	0.04	0.91	45	
36	0.001	0.75	1.65	2.00	1.65	1.65	1.40	1.25	10.35	517	77
	0.05	11.00	0.88	0.44	0.34	0.29	0.25	0.20	13.40	670	
37	0.001	1.10	1.15	1.10	1.10	1.10	1.10	1.10	7.75	387	59
	0.05	10.80	1.60	0.31	0.20	0.16	0.12	0.08	13.30	663	
38	0.001	0.40	0.80	1.24	1.44	1.16	0.50	0.40	5.94	297	87
	0.05	4.00	1.00	0.48	0.31	0.25	0.20	0.16	6.40	320	
64	0.001	0.68	1.60	1.60	1.44	0.88	0.88	0.88	7.96	398	56
	0.05	8.00	3.20	0.80	0.70	0.60	0.40	0.40	14.10	705	
80	0.001	0.88	1.76	2.00	2.00	1.60	1.10	1.00	11.00	550	60
	0.05	16.00	2.90	0.20	0.10	0.10	0.06	0.04	19.40	970	
35	0.001	0.02	0.04	0.04	0.04	0.04	0.06	0.07	0.31	15	13
	0.05	0.66	0.44	0.29	0.25	0.25	0.22	0.20	2.31	115	
59	0.001	0	0	0	0	0	0	0	0	0	0
	0.05	0.10	0.04	0.01	0.01	0.01	0	0	0.17	8	
89B	0.001	1.16	2.00	1.36	0.80	0.60	0.54	0.40	7.06	353	44
	0.05	7.20	2.20	1.40	1.30	1.30	2.00	0.90	16.20	810	

voir of dilute acid is kept filled. When one soil is sufficiently extracted, it may be quickly replaced by another, a new set of collecting bottles is set in position and the process continues without further attention.

It is obvious that in order to have truly comparable results with different soils, all variables except the soil, should be kept out of the procedure. This is fairly well done by the apparatus used. It has been more fully described in another paper (13). The rate of flow of the dilute acid through the soil varies somewhat from moment to moment, but averages very nearly 1 liter every

TABLE 10

Analyses of soil percolates made with 0.001 N *HCl*

PERCOLATE NUMBER	pH	Ca	PO_4	pH	Ca	PO_4	pH	Ca	PO_4	pH	Ca	PO_4
		p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
	Soil 38			*Soil 64*			*Soil 35*			*Soil 59*		
1	4.0	14	0.16	4.0	14	0.08	3.6	12	0	4.0	20	0
2	3.8	14	1.08	4.0	14	1.32	3.4	16	0	3.6	6	0
3	3.6	22	1.44	4.2	14	2.00	3.3	10	0	3.2	3	0
4	3.4	18	1.44	3.8	14	1.60	3.2	6	0	3.2	0	0
5	3.4	10	1.08	3.4	10	1.32	3.2	2	0.04	3.0	0	0
6	3.2	6	0.56	3.2	6	1.00	3.0	1	0.07	3.0	0	0
7	3.2	6	0.48	3.0	2	0.80	3.0	0	0.07	3.0	0	0
	Soil 1C			*Soil 30*			*Soil 37*			*Soil 80*		
1	6.2	4	1.50	4.6	12	5.00	6.8	5	1.10	5.2	6	0.88
2	5.6	5	2.65	3.3	1	4.40	6.2	7	1.15	5.2	6	1.76
3	4.4	7	2.80	3.2	0	2.70	5.4	12	1.10	4.2	14	2.00
4	4.2	7	2.65	3.2	0	2.00	5.4	15	1.10	3.8	10	2.00
5	4.0	7	2.50	3.0	0	1.10	5.4	12	1.10	3.4	6	1.60
6	3.7	4	2.00	3.0	0	0.95	5.4	10	1.10	3.0	3	1.10
7	3.4	3	1.65	3.0	0	0.90	5.2	8	1.10	3.0	tr.	1.00
	114 mgm. $CaHPO_4$, $2H_2O$			*66 mgm. $Ca_3(PO_4)_2$*			*0.2 gm. white rock phosphate*			*0.2 gm. black rock phosphate*		
1	3.6	..	66.00	3.4	..	40.00	3	6	15.6	3.4	15	25.0
2	3.0	..	6.20	3.0	..	0.36	3	4	10.8	3.0	12	21.0
3	3.0	..	0.96	3.0	..	0.30	3	3	7.3	3.0	8	15.4
4	3.0	..	0.34	3.0	..	0.24	3	..	6.6	3.0	..	13.5
5	3.0	..	0.26	3.0	..	0.20	3	..	6.3	3.0	..	7.3
6	3.0	..	0.24	3.0	..	0.18	3	..	5.6	3.0	..	5.2
7	3.0	..	0.22	3.0	..	0.20	3	..	5.3	3.0	..	3.1

three hours. Starting with 20 gm. of soil, nearly all the PO_4 soluble in 0.001 *N* acid is extracted from good soils by 7 liters of percolate, but not all is removed from some of the soils having the PO_4 in less available condition. The rate of flow being nearly constant, and the aliquots of percolate being of the same volume, the results for different soils are thought to be properly comparable. The figures obtained for a number of soils are set out in table 9, which also

gives the figures found when 0.05 N acid was used. The figures reveal the great difference between soils as to solubility of PO_4. Some, such as 1C, 36, 38, 65, have considerable buffer power so that much of the free hydrogen ion of the first liter of acid passing through the soil is neutralized with the result that there is little change in pH of the soil and consequently little PO_4 dissolved. As the buffer effect of the soil is overcome, pH is lowered and PO_4 in the percolate increases to a maximum, which depends on the rate of flow and the

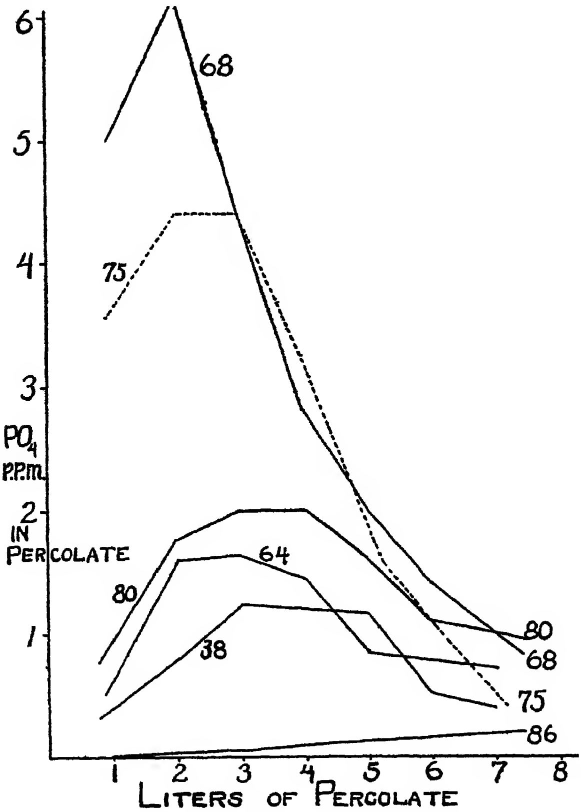


FIG. 2. PO_4 IN EXTRACTS MADE BY CONTINUOUS PERCOLATION WITH 0.001 N HCl

character of the soil. Other soils, such as 30, 53, 68, have little buffer power and this is soon overcome by the dilute acid so that the highest concentration of PO_4 is found in the first liter of percolate, and rapidly decreases thereafter. At first, it was thought that this indicated how the soils would respond in supplying PO_4 to a growing crop. Experience has not yet shown whether or not this is true.

In the use of this method, it is a very simple matter to determine Ca by tur-

(1931–32)

bidity and pH by color comparison in the separate bottles of percolate. From 1 to 2 hours' time is enough to make all the tests for two soils, in the 14 bottles. This information often helps much to understand the nature of the soil. Table 10 presents some of the figures thus found. Most of the easily soluble or replaceable Ca, presumably Mg also, is soon removed along with the easily soluble PO_4. When the soil contains much $CaCO_3$, this neutralizes the acid and prevents lowering of pH, so PO_4 is not so rapidly extracted. This is shown by soil 37 which contains 1 per cent $CaCO_3$. Some soils such as 35 and 59 contain a fair amount of PO_4, but it is practically insoluble in 0.001 *N* acid. In such soils, usually the amount found in the percolate increases slowly for some time as the volume of percolate increases. In these, it seems probable that the PO_4 is combined with Fe or Al in the soil in such manner that it is not soluble in 0.001 *N* HCl. When the PO_4 is combined with Ca, it is easily dissolved by this acid (table 10, in which the action on $CaHPO_4$ and $Ca_3(PO_4)_2$ and on two rock phosphates is shown). In this work, HCl is the acid used because of its convenience and the easy solubility of its reaction products. HNO_3 works in the same way and is more to be expected in the soil solution, but it is likely to interfere in the colorimetric estimate of PO_4. HCl of 0.001 *N* strength has a pH of about 3, somewhat similar to that of water saturated with CO_2 and perhaps not greatly different from the pH of the sap of some plants. It has very little action on soil silicates or zeolites except to replace imperfectly Ca, Mg, Na and K.

Percolation with 0.05 N *HCl.* This concentration of acid was selected because it seems strong enough to remove from the soil all the PO_4 that plants are likely to be able to extract within many years. It almost completely replaces bases, Ca, Mg, Na, and K. It completely extracts PO_4 from $Ca_3(PO_4)_2$ and from ordinary rock phosphates. It dissolves some iron and silica, usually very little from ordinary soils. In order to carry the extraction of PO_4 to completion with 0.05 *N* acid, the dissolved substances must be removed so that the reaction can go to completion in one direction. This is accomplished by continuous percolation.

The concentration of hydrogen ion in this acid, about pH 1.35, is sufficient to overcome quickly the buffer power of 20 gm. of ordinary soil so that the larger portion of the available PO_4 is removed by the first liter of percolate. Succeeding liters of percolate contain less and less PO_4, as shown in table 9. On account of the rapid removal of the PO_4 by this acid, little useful information is obtained by testing each liter of percolate separately, so the whole is collected together. Some relatively infertile soils still give up appreciable amounts of PO_4 to 0.05 *N* HCl after 7 liters have percolated through 20 gm. of soil at the rate of 200 to 300 cc. an hour. This is true of soils 35 and 64, and of 59 to which considerable amounts of superphosphates have been added. Very little PO_4 is extracted from untreated soil 59. In many cases the amount of PO_4 extracted by percolation with 0.05 *N* HCl fairly well represents the capacity of the soil to supply PO_4 to crops, but perhaps not as well as the 0.001 *N* percolate.

Since carbonic acid is perhaps the most important in its effects of any free acid usually found in the soil, it would seem reasonable to use this agent for extracting PO_4 from the soil. For this purpose, a modification of the apparatus used for percolating with 0.05 N HCl was made in order to use water saturated with CO_2 under a head of about 1.5 m. of water. The gas was caused to bubble into the lower end of the column of water which was flowing to the percolator. From the top of this column, the CO_2 passed into the reservoir which supplied the water so that the water was kept saturated with the gas at all times, at that temperature and pressure. The pH of this solution as it passed to the soil in the closed percolator was about 3, like that of 0.001 N HCl, but the total acid-

TABLE 11

PO_4 extracted from soils by various solvents, and by different procedures

SOIL NUMBER	TOTAL BY FUSION	PO_4 IN SOIL				EQUILIBRIUM WITH WATER, 1:1	PO_4 IN SOLUTION AT 1:5 EQUILIBRIUM WITH ACIDS						
		By hot HCl, 10 per cent	By percolation with				Normality of HCl				Normality of citric acid		
			0.05 N HCl	0.05 N CO_2	Water, pH 5		0.01	0.02	0.20	1.0	0.025	0.05	0.20
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1C	2,000	1,160	680	660	307	0.18	4	10	50	200	11.4	27	66
30	1,600	1,370	1,197	702	290	8.00	40	80	166	310	35	40	88
35	2,400	1,120	140	0	0	0.12	0.16	0.24	10	44	5.6	8.8	11
36	2,500	1,020	418	420	...	0.30	2.3	9.4	36	160	13	20	36
37	2,400	.....	494	829	...	0.47	0	0.2	25	160	5	....	44
38	2,500	990	320	210	40	0.22	0.8	1.2	13	57	10	13	24
40	3,300	2,720	1,430	1,100	...	1.40	13	20	166	360	33	....	160
45	3,800	.....	1,850	.....	...	0.40	5	13	.....	...	13	....	.....
46	1,600	.....	80	.....	...	0	1.2	2.2	.....	...	5.6	....	.....
59A	2,000	100	tr.	.....	...	0	0	0	0.3	1	0.6	0.7	2
64	1,700	1,180	650	360	63	0.16	0.4	2	50	100	10	13	27
65	2,800	.....	1,170	1,170	...	3.4	35	60	200	280	80	....	182
69A	1,600	1,210	12	0	...	0.14	0.3	0.4	1.4	100	2.3	....	3.2
78A	450	340	50	13	...	0.20	1.4	1.8	8	22	....	....	6.6
80	1,150	950	795	738	128	0.30	2.3	27	118	200	....	....	....

ity, found by titration, was about 0.05 N, like that of the HCl used in the same way.[14] In other words, little of the CO_2 was ionized, the solution was highly buffered, and its solvent power approached that of 0.05 N HCl. In table 11, column 5, are given a few figures showing the results obtained by the use of this acid. Since it is very inconvenient to use, and the results it gives are no more significant than those obtained with HCl, its use was not long continued. Although the water solution of CO_2 entering the percolator had a pH of about 3, the solutions coming from the percolator, when the pressure no longer confined the gas, had a pH near 5.

More dilute solutions of CO_2 in water were tried but their solvent power was so little that they seemed impractical. Water containing CO_2 at pH 5, which

is about that of ordinary distilled water, must be percolated through a mass of 20 gm. of good soil for many days in order to extract as much PO_4 as will be removed by 7 liters of 0.001 *N* HCl in 15 hours (table 12). For the practical purpose of estimating the easily available PO_4 in a soil, water containing none or only small amounts of CO_2 is very ineffective. A greater concentration of hydrogen ion than can be had in this way is necessary to produce much solvent effect.

In table 11 are presented the results of analyses of several different soils by a number of different procedures with various solvents of different strengths. There are three subdivisions of the table; first, the total PO_4 found by fusion, and by hot 10 per cent HCl; second, PO_4 found by percolation; and third, the PO_4 found in equilibrium extracts. The figures are thus assembled to facilitate comparisons of the different methods. In most cases, the total PO_4 found by fusion and that extracted by strong hot acids, has little relation to the value of the soil as shown by plants, so that such determinations are generally useless

TABLE 12

*PO_4 extracted by percolating with ordinary distilled water**

SOIL NUMBER	PO_4 IN SOIL
	p.p.m.
1C	307
30	290
35	0
38	40
64	63
80	128

* The same apparatus and procedure were used as with 0.05 *N* HCl.

Volume of percolate, 6–7 liters. In all except 35, there was still much PO_4 in the last percolate, showing that the soil was not nearly exhausted of water-soluble PO_4 by this method.

for indicating the fertility of the soil. Equilibrium extracts with dilute weak acids supply much more useful figures. The best indication is the result found by percolation with very dilute acids. This will be considered in more detail later.

Relative solubilities with different solvents. The amount of PO_4 extracted from soil by any of the aforementioned methods, either by equilibrium or by percolation, does not always indicate whether or not the PO_4 thus obtained will be supplied to plants by the soil in such manner or rate that it will support good growth of crops. Other students of this subject have reached a similar conclusion. To provide a more reliable or adequate notion of availability, the factor "relative solubility" has been introduced. One of its most prominent advocates is Lemmermann (14), who has contributed numerous papers on this subject. He extracts soil with hot 10 per cent HCl and with 1 per cent citric acid. When the PO_4 extracted by the latter is less than 25 per cent of that dis-

solved by the HCl he considers that the soil is likely to respond to phosphate fertilizers. In this laboratory, a number of soils treated by this method gave results not in accord with their behavior in pot cultures. It may be that the amount and concentration of the acids used by Lemmermann are not well adapted to our soils. The principle seemed worthy of further study. It was applied to a number of combinations of different solvents without giving results of much value, except in one case. The results are given in table 13.

The Lemmermann numbers shown in this table in the column headed 100 C/B show some of the soils in their proper relations, but others are far from correct; e.g. 59, 64, 78, the poor soils, are made to appear better than they really

TABLE 13

Relative solubilities of soil phosphate

SOIL NUMBER	RELATIVE SOLUBILITY		
	$\frac{100\ C}{B^*}$	$\frac{100\ D}{A}$	$\frac{100\ E}{D}$
1C	5.7	48.0	82
30	6.4	70.0	76
35	1−	6.0	7
36	3.5	27.0	77
38	2.4	13.0	87
40	5.8	43.0	60
53	5.6	57.0	91
59	2.0	0.0	0
64	2.3	39.0	56
69	0.2	0.7	0
78	2.0	10.0	71
80	...	84.0	60

* 100 *C/B* is Lemmermann's factor of availability.
A = p.p.m. PO_4 in soils found by fusion (see table 11).
B = p.p.m. PO_4 in soils found by equilibrium hot 10 per cent HCl (see table 11).
C = p.p.m. PO_4 in soils found by equilibrium 0.2 *N* citric acid (see table 11).
D = p.p.m. PO_4 in soils found by percolation with 0.05 *N* HCl (see table 9).
E = p.p.m. PO_4 in soils found by percolation with 0.001 *N* HCl (see table 9).

are. In the column headed 100 D/A the valuations are more nearly right, although in this, soils 38 and 64 are not in proper relation to each other. In the column headed 100 E/D, 38 and 64, as well as all the others, receive correct values.

Relative solubility of soil PO_4 in 0.001 N *and in 0.05* N *HCl, by percolation.* In all cases so far observed, it is found that if the PO_4 extracted by percolation with 0.001 *N* HCl is less than half as much as is found by similar treatment with 0.05 *N* HCl, the soil is likely to be deficient in power to supply phosphate to plants. On the other hand, good soils give up nearly as much PO_4 to the weaker as to the stronger acid. The results for a number of soils are given in table 9,

in the last column of which is shown the percentage of PO_4 by percolation with 0.001 N HCl of the amount found by 0.05 N HCl. This gives soils 64 and 80 a low ratio of availability of PO_4, although the amounts extracted are higher than for soil 38, which gives good results in pot cultures without added PO_4, whereas 64 and 80 require phosphate in order to produce good crops. When the absolute amount of PO_4 extracted by either of these acids is very low, as in soil 78A, crops respond to added phosphate even though the relative solubility of that originally present is high.

TABLE 14

Phosphate-supplying power of soils

As related to particle size—per cent clay × p.p.m. PO_4 extracted with 0.05 N HCl by percolation

SOIL NUMBER	CLAY	PO_4	CLAY × PO_4
	per cent	*p.p.m.*	
1C	42	680	28,560
21	5	525	2,625
30	7	1,197	8,400
35	29	140	3,060
36	30	418	12,540
37	39	494	20,000
38	52	320	16,640
40	31	1,430	44,330
44A	40	100	4,000
53A	4	500	2,000
54A	42	175	7,350
55A	37	860	31,820
56A	40	490	19,600
59A	48	tr.	48
64	10	350	3,500
65	32	1,170	37,440
66A	42	6	288
68A	12	1,100	13,200
69A	50	12	600

Amount of phosphate in various sized particles

An attempt to explain the relative fertility of soils 38 and 64 on the basis of the amount of PO_4 in large and small sized particles gave no useful information. The soils were separated into sand, silt, and clay fractions by sedimentation in water. The PO_4 in the various fractions was determined but the figures obtained were so similar for the two soils that the differences seemed inadequate to explain the great difference between the two soils respecting available phosphate as indicated by the growth of plants.

It is evident that the physical texture of a soil may greatly affect the availability of the soil phosphate. A fine textured soil has vastly more points of contact for plant roots than a coarse soil, so that a much lower concentration of

PO_4 in the fine soil may furnish a more adequate supply to plants than would a coarse soil having a much higher content of PO_4 in its larger particles.

Failyer, Smith and Wade (10) indicate that in most of the soils examined, the concentration of PO_4 in various sized particles is greater as the size of the particles is smaller.

An attempt was made to correlate the availabiity of soil phosphate with the proportion of fine material in the soil. The percentage of clay in the soil was multiplied by the number of parts per million of PO_4 found by percolation with 0.05 N HCl. In general, the higher the figure thus obtained, the greater is the amount of avilable PO_4 in the soil. But the availability of PO_4 in some sandy soils such as 30 and 68, is much underestimated by this method, whereas soils 38 and 64 receive a relatively proper value. The figures obtained by this calculation are shown in table 14.

TABLE 15

Comparison of phosphate extracted by equilibrium and by percolation

SOIL NUMBER	PO_4 IN SOIL					
	Equilibrium extracts, 1:5 with			1:10 with 0.2 N HNO_3, Fraps method	Percolation extracts, with HCl	
	0.02 N HCl	0.20 N citric	1.0 N HCl		0.001 N	0.05 N
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1C	50	66	200	400	787	965
30	166	88	310	1,120	855	1,115
35	10	11	44	80	15	115
36	36	36	160	220	517	670
37	25	44	160	250	387	663
53	100	44	160	500	417	458
59	0.3	2	1	2	0	8
38	13	24	57	80	297	320
64	50	27	100	270	398	705

Relative amounts of phosphate extracted from soils in equilibrium extracts compared with amounts found by percolation with different acids

The data shown in tables 11 and 12 permit a comparison better shown by recombination of the figures in table 15.

It is apparent that the amount of PO_4 extracted increases as the strength of the acid is increased and that very much more PO_4 is obtained by percolation with the dilute acids than in equilibrium extracts made with much more concentrated acids. Exceptions are soils 35 and 59A which have relatively very little available phosphate. Such soils would show no available PO_4 to the dilute acid used in percolation if it were not for the large volume of acid used in percolation.

It appears to be a general law with respect to solubility of soil phosphate that PO_4 dissolved increases as concentration of hydrogen ion increases, and also as the volume of solvent is increased.

From table 11, it may be seen that there is no relation between the total PO_4 in a soil and the available PO_4 extracted by dilute acids.

Effect of several salts on solubility of soil phosphates

To 100 cc. of water were added 50 gm. of soil and 1 gm. of the salt. After being shaken one hour, the mixture was filtered and the filtrate tested for PO_4. Soils 1C, 36, 37, 38 were used in the experiments. When $FeSO_4$, $CuSO_4$, and $CuCl_2$ were used, no PO_4 was found in the filtrates.

With NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3, $CaCl_2$, $CaSO_4$, $BaCl_2$, $NaCl$, Na_2SO_4, KCl, and K_2SO_4 there was about as much PO_4 in the extracts as with distilled water alone. Ammonium oxalate and ammonium citrate gave extracts containing about 10 times as much PO_4 as did distilled water.

There seemed to be no useful information obtained in this way, so the study was not long continued.

METHODS PROPOSED BY OTHERS

In the course of this study, several methods proposed by others have been studied briefly. The work of von Wrangell and collaborators (27, 28, 29, 30, 31) is most instructive. Time and suitable equipment have not been available to the writer to repeat or verify the very interesting, painstaking, and fundamental work of these investigators in respect to soil phosphates and their availability to plants.

Von Wrangell's work

Shortly after the main group of papers from these workers were published, there appeared in *Chemical Abstracts* brief mention of them, but these abstracts did not make evident the importance of the work done. Indeed, it seems probable that few except German workers in this field have yet heard of, or become impressed by, this work. The writer did not know of it until after the work here reported was done. The conclusions of von Wrangell seem to be supported by such work done in our laboratories as has any bearing on the subject.

The chief factors to be considered in evaluating the ability of a soil to supply PO_4 to growing plants have been very well ennumerated by von Wrangell (27, p. 637): (*a*) The kind of plant, (*b*) presence of lime salts, (*c*) effects of electrolytes and organic substances in the soil, (*d*) the reaction and absorbtive power of the medium in which the plant grows, (*e*) action of microflora. Also it is important to know the time rate at which a soil will regain its original concentration of PO_4, after it has been depleted by extraction with water or by growing plants.

In executing the von Wrangell method, 1 gm. of soil is shaken five hours with 100 cc. of water. The solution is clarified by centrifuging, the clear fluid is siphoned off, and the soil is again shaken with 100 cc. of water as at first, and thus a second extract is obtained. Sometimes further extractions are made in the same manner.

The PO_4 found in the two extracts is designated a and b, then by the formula $a^2/a\text{-}b = x$, is found the total available PO_4 in the soil. If the necessary machines are available the method is short, simple, and convenient. Prof. von Wrangell states that the results obtained are in good accord with the results of pot cultures and field tests.

The writer attempted to obtain similar results by separating the soil extract by filtering, as a suitable centrifuge was not available. This makes the method tedious and less exact than that proposed by von Wrangell, but the results seemed to agree, for most of the soils tried, with the known character of the soils in respect to capacity of the soils to supply phosphate to plants. If this method is found by general experience to prove as reliable as its author predicts, it will, perhaps, replace most other methods now in use.

For estimating the availability of phosphate in calcareous soils, Das (6) has suggested the use of potassium carbonate. Such a reagent may hydrolyze iron and aluminum phosphates, making PO_4 soluble, also replacing Ca in $CaHPO_4$, thus bringing PO_4 into solution. Such an alkaline reagent also dissolves much organic matter, including some organic phosphorous. It is difficult to separate organic from inorganic phosphorous, also the solution is troublesome to prepare for analysis by the molybdenum blue method. Some trials of the method in this laboratory indicated that the results obtained by it have little relation to availability of the phosphate in nearly neutral soils.

The Neubauer (15) method for determining the availability of soil phosphate has not been investigated during this study. It is a plant culture, not a chemcal method. A good comparison of the Neubauer and Lemmermann methods is given by Engels (9) and by Blanck (3). In this country, Neubauer's method for potassium has been studied by Ames and Gerdel (1), and by Haley and Holben (12). As applied to phosphate, there seems to be no published report of work with the method in this country.

Arrhenius (2) proposes to extract the soil with a 1 per cent solution of NaCl containing enough H_2SO_4 to make the solution about 0.002 N in hydrogen ion. As might be expected, the writer found that the PO_4 thus extracted is about the same as if the dilute acid alone were used. This method does not give proper relative values to the power of the soils examined to supply plants with available PO_4.

Dirks and Scheffer (7) add to 100 gm. of soil, 1 gm. $CaCO_3$ and 250 cc. water, then pass CO_2 through the mixture. After some hours, PO_4 is determined in the filtrate. The results obtained in this laboratory by this method were not in agreement with the results shown by plants on the same soils.

Christensen (5) has proposed the use of Azotobacter as an indicator for availability of soil phosphate. His method has been modified and used by Niklas and others (16, 17, 18). A trial of it in this laboratory failed to give useful results. Yet Winogradsky (26) says it gives reliable results in 85 per cent of the tests.

Dyer (8), in 1894, proposed 1 per cent citric acid as an agent for determining available soil PO_4. Much useful information has been obtained in this way, yet the results are not always satisfactory.

Many investigators [notable among them is Fraps (11)] have used 0.2 *N* HNO_3 for determining the availability of soil phosphate. Some have used much stronger acids, e.g. Lemmermann who used hot 10 per cent HCl compared with 1 per cent citric acid to give his factor for "relative solubility" as already explained (p. 455). To the writer, it seems that the use of so drastic a reagent as hot 10 per cent HCl to extract available PO_4 from soil is unlikely to indicate the behavior of the soil with growing plants.

The opposite extreme is found with those who use water only to determine PO_4 is soil. Various ratios of soil to water have been tried, all the way from the displaced soil solution as obtained by Burd and Martin (4), to the 1 to 100 ratio used by von Wrangell. Undoubtedly the soil solution indicates what the plant has to deal with at the time the extract is made, but since the soil solution may change from day to day, it does not show what the plant can obtain from the soil over a considerable period of time. This might be expected, since only a small amount of soil phosphate which is available to plants from soils having a good supply of available phosphate, is dissolved in the soil solution at any one time. The same is probably true in regard to soil extracts made with larger ratios of water to soil, unless it be that when the ratio is wide, as in the method of von Wrangell (1 soil to 100 water), all the easily available PO_4 is brought into solution.

Meyer, a collaborator with von Wrangell (31), obtained somewhat useful results in this laboratory in 1927 by making repeated extractions of the same small portions of soil with 100 times as much water, a variation of the von Wrangell method. The plotted results gave a curve indicating fairly well the availability of the soil phosphate.

At about the same time, J. W. Tidmore in this laboratory made successive diffusions of a small amount of soil in collodion bags suspended in water, by the method of Pierre and Parker (20). The amount of PO_4 found in the successive diffusates was plotted to produce a curve which indicated fairly well the ability of the soil to supply phosphate to plants. The process was prolonged and tedious, though not requiring a great deal of time or attention from the operator. Much time was required because a considerable fraction of the PO_4 dissolved by the water remained in the colloidon sack with the soil each time when the diffusate was removed from the outside container. Although the figures obtained by Meyer and by Tidmore in most cases gave a useful indication of the power of the soil to supply phosphate to plants, the results were not always in accord with the growth of plants in those soils as related to available phosphate.

Apparently no one of the published methods has been found always reliable, so the search still continues.

CONCLUSIONS

In the opinion of the writer, a satisfactory estimation of the availability of soil phosphate by extraction with any one solvent of whatever strength will not, in some cases, be possible. Relative solubility and the total PO_4 dissolved in two solvents of quite different strength, but neither of them strong enough to cause much decomposition of the soil, provide figures much better indicating the capacity of the soil to supply plants with phosphate.

A fairly reliable estimate of the phosphate-supplying power of an unknown soil may be most easily and quickly obtained by analysis of the extract made by the aid of a dilute acid which has been added in such amount that the extract will have approximately pH 4. When there is some uncertainty in regard to the availability of the phosphate thus found, the more certain, as well as more laborious, method of "relative solubility" should be applied.

The equilibrium test will at once indicate the very poor and the very good soils, with the least time and expense. The percolation tests will give more reliable information in uncertain cases.

The only certain means of determining the response of a soil to fertilizers lies in the actual trial with plants in greenhouse or field.

SUMMARY

Some reasons why a satisfactory estimation of the availability of soil phosphates by chemical methods is difficult are:

Phosphate found in soils is mostly present in relatively slightly soluble combinations, so that the concentration in the soil solution is never high.

Plants have selective action in absorption of nutrients, and also it seems probable that plants are able to take up ions such as PO_4, K, perhaps others from films of solution not represented by solutions prepared in the laboratory.

No chemical agent can imitate these effects of plants on soils.

Many soils have such high fixing power that an easily soluble phosphate added to them is quickly fixed, thus becoming more or less unavailable to plants.

Deficiency of other necessary plant nutrients in the presence of sufficient phosphate, or presence of excessive amounts of soluble salts or toxic substances in the soil, may cause the failure of plants.

Test of a soil some time after phosphate has been added and after plants have grown on it subsequent to addition of the phosphate cannot show the condition of the soil with which the plant had to deal at the start.

Equilibrium, 1:5 extracts of soils with dilute acids provide the simplest and quickest means of obtaining some idea of the available phosphate, although such extracts do not always give a correct idea of the relative power of different soils to supply phosphate to plants. Extracts made with three or more different concentrations of an acid give a much better picture of the available PO_4 than is obtained by any single extract. Extracts thus made show only the PO_4 available at the time of extraction, not the relative supplying power of the soil for any great length of time.

Citric and oxalic acids, because they form soluble complexes with Fe, Al, Ca, and Mg (the cations which tend to repress solubility of PO_4), are not appropriate for determining the availability of soil phosphates.

A solution of CO_2 in water would be very appropriate for the purpose if it were easily possible to duplicate conditions at will, but its application is too difficult.

Dilute K_2CO_3 hydrolyzes some phosphates and thus brings PO_4 into solution, but the results do not seem to have much bearing on availability.

If results of tests of different soils are to be compared, the buffer power of the soil must be reckoned with. The amount of acid used in making an extract should be varied according to the buffer power of the soil so that the extracts of all soils will have the same pH, e.g. 4. This means that some soils will need two or three times as much acid as others to have an extract with pH 4.

Since it is difficult to prepare a soil extract of exactly pH 4, or other definite figure, the PO_4 dissolved at any such definite point may be found exactly, by making three extracts with three different concentrations of acid, plotting the results and from the graph finding the PO_4 at the desired pH. For this purpose, a highly buffered acid is desirable. Acetic is very appropriate, much better than HCl.

The amount of PO_4 dissolved increases as the volume of solvent is increased, up to a large dilution. Water extracts of some soils have almost the same concentrations of PO_4 regardless of the volume of water used, up to a dilution of 100 or more water to 1 soil.

The results of such equilibrium extractions seem to be more in accord with plants' response the nearer the proportion of water to soil is to that in the actual soil. But since such extracts are difficult to prepare, a ratio of 1 soil to 5 water serves very well and is more convenient.

Vanstone's "rate of solubility curve" does not in all cases express the plant availability of soil phosphate. It seems to offer little advantage over other equilibrium methods.

Percolation methods of making soil extracts resemble the action of a plant more than equilibrium methods, yet do not show the power of the oil to continue supplying PO_4 so well as was expected and hoped. In these methods the soil is percolated slowly with very dilute acid till most of its soluble PO_4 is removed. An automatic apparatus for percolation has given very good results. Curves formed by plotting the results from percolation extracts are characteristic of the soil's individual power to supply phosphate.

Percolation with 0.05 N HCl is supposed to dissolve from a soil all the PO_4 that may be expected to be avilable to plants for many years.

Carbonic acid, 0.05 N, has much the same solvent power as HCl, but is very inconvenient.

Water alone, or containing only a little CO_2, is so poor a solvent for soil PO_4 that it seems inadequate as an agent for estimating availability by percolation methods.

Relative solubilities. No single method of extracting PO_4 from soil, whether by equilibrium or by percolation, has been found always adequate to show whether a soil will supply phosphate to plants in sufficient amount and at a rapid enough rate to support satisfactory plant growth. But the *relative solubility* in two reagents of different power seems to give a figure which indicates very well the phosphate value for many soils. When the PO_4 found by percolation with 0.001 N HCl is 60 per cent or more of the amount found by percolation with 0.05 N HCl, the soil will supply plant growth adequately, provided the total found by 0.001 N HCl is 300 p.p.m. or more. When the PO_4 extracted by the more dilute acid is less than 50 per cent of the amount dissolved by the 0.05 N acid the soil is likely to be deficient in supporting plant growth even though the total amount found by the 0.05 N acid is adequate, provided it were available. The greater the difference in the amounts extracted by the two different concentrations of acid, the more likely is the soil to be deficient in supplying phosphate.

In general, more of the PO_4 in soils is contained in the fine than in the coarse particles, and the finer the particles the more available the PO_4 will be to plants. But when the percentage of clay and silt in soils was multiplied by the number of parts per million of PO_4 found in the soil, the figures did not always properly represent the PO_4 supplying power of the soil.

PO_4 dissolved from soil by an acid increases as the pH of the soil decreases and as the ratio of solvent to soil increases.

In making equilibrium extracts 1 per cent of a number of common salts added to the water did not much change the amount of PO_4 dissolved. Salts of iron and copper greatly decreased the PO_4 dissolved.

The method proposed by von Wrangell appears very promising so far as the writer has been able to test it, which is very little.

The methods proposed by Arrhenius, extraction with 1 per cent NaCl in 0.002 N H_2SO_4; by Dirks and Scheffer, extraction with CO_2 and $CaCO_3$; by Christensen's Azotobacter method; by Dyer's 1 per cent citric acid extract; by Fraps' 0.2 N HNO_3 method; by water extracts with varied proportions of soil to water; and by Lemmerman's "relative solubility" method; have been examined and none of them found generally successful with all kinds of unknown soils, though all of them may give quite useful information when applied to the kind of soil for which they were designed.

Successive water extracts made by the method of diffusion with collodion bags give a much better indication of the continuous supplying power of the soil than do single equilibrium extracts but do not with certainty give proper relative values to all soils.

REFERENCES

(1) Ames, J. W., and Gerdel, R. W. 1927 Potassium content of plants as an indicator of available supply in soil. *Soil Sci.* 23: 199–216.

(2) Arrhenius, O. 1929 The phosphoric acid question in the cultivation of sugar cane in Java. *Chem. Abs.* 23: 2780.

(3) BLANCK, E. 1926 A contribution to the determination of the soil's need for fertilizer. *Ztschr. Pflanzenernahr. Düngung. u. Bodenk.* (B) 5: 118; also *Jour. Landw.* (1928). 75: 343–52.

(4) BURD, J. S., AND MARTIN, J. C. 1923 Water displacement of soils and the soil solution. *Jour. Agr. Sci.* 13: 265–295.

(5) CHRISTENSEN, HARALD 1915 Studies on the influence of soil conditions on bacterial life. *Centbl. Bakt.* 43 (II): 1–166.

(6) DAS, S. 1926 Determination of PO_4 in calcareous soils. *Chem. Abs.* 20: 2038.

(7) DIRKS, B., AND SCHEFFER, F. 1928 Rapid methods for the determination of the phosphorus requirements of soils. *Landw. Jahrb.* 67: 779.

(8) DYER, BERNARD. 1894 Analytical estimation of probably available mineral plant food in soils. *Trans. Chem. Soc.* 65: 115–167.

(9) ENGELS, O. 1926 Comparison of the methods of Neubauer and Lemmermann. *Ztschr. Pflanzenernähr. Dungung. u. Bodenk.* (B) 5: 537.

(10) FAILYER, G. H., SMITH, J. G., AND WADE, H. R. 1908 The mineral composition of soil particles. U. S. Dept. Agr. Bul. Soils Bul. 54.

(11) FRAPS, G. S. 1909 Active phosphoric acid and its relation to the needs of the soil for phosphoric acid in pot experiments. Texas Agr. Exp. Sta. Bul. 126.

(12) HALEY, D. E., AND HOLBEN, F. J. 1927 A biological measurement of available soil potassium. *Soil Sci.* 24: 345–350.

(13) HIBBARD, P. L. 1930 An aparatus for percolation at a uniform constant rate, etc. *Indus. and Engin. Chem.* Anal. Ed. 2: 404.

(14) LEMMERMANN, O. 1923, 1926 Methods for determination of relative solubility of soil phosphate. *Ztschr. Pflanzenernähr. u. Dungung.* (A) 2: 363–70; (B) 5: 105–118.

(15) NEUBAUER, H., AND SCHNEIDER, W. 1923 Food consumption of germinating plants and their use in the determination of the fertilizer requirements of soils. *Ztschr. Pflanzenerndhr. u. Düngung.* (A) 2: 329–362.

(16) NIKLAS, H., AND HOCK, A. 1927 The biochemical Azotobacter method for testing the phosphate needs of soil. *Ernähr. Pflanze.* 19: 297–300.

(17) NIKLAS, H., SCHARRER, K., AND STROBEL, A. 1926 Phosphate solubility and growth of Azotobacter. *Landw. Jahrb.* 63: 387–410.

(18) NIKLAS, H., AND HIRSCHBERGER, W. 1925 A new method for the rapid determination of the phosphoric acid requirements of our soils. *Chem. Abs.* 19· 1610.

(19) PARKER, F. W., AND FUDGE, J. F. 1927 The colorimetric determination of organic and inorganic phosphorus in soil extracts and in the soil solution. *Soil Sci.* 24: 109–117.

(20) PIERRE, W. H., AND PARKER, F. W. 1927 The use of collodion sacks in obtaining clear soil extracts for the determination of water-soluble constituents. *Soil Sci.* 23: 13–32.

(21) RUSSELL, E. J. 1921 Soil Conditions and Plant Growth, p. 120. Longmans, Green and Co. London.

(22) SIMON, R. H. 1930 Effect of phosphate and lime upon the rate curve of solubility of a Wooster silt loam soil. *Soil Sci.* 29: 71.

(23) TEAKLE, L. J. H. 1928 Phosphate in the soil solution as affected by reaction and cation concentration. *Soil Sci.* 25: 143–162.

(24) TRUOG, E. 1930 The determination of readily available phosphorus in soils. *Jour. Amer. Soc. Agron.* 22: 874.

(25) VANSTONE, E. 1925 Available phosphate in soils. *Jour. Agr. Sci.* 15: 460.

(26) WINOGRADSKY, S. 1928 Note on the Azotobacter test for available phosphate in soils. *Compt. rend. Acad. Sci. (Paris).* 187: 161–165.

(27) Wrangell, M. von 1926 I. Soil phosphate and need for phosphate. *Landw. Jahrb.* 63: 627–668; II. Colorimetric method for determination of PO_4. *Ibid.* 63: 669–675.

(28) Wrangell, M. von 1930 III. The determination of available plant nutrients in the soil. *Ibid.* 67: 149–169.

(29) Wrangell, M. von and Haase 1926 IV. Phosphate content of natural soil solution. *Ibid.* 63: 707–738.

(30) Wrangell, M. von and Koch 1926 Solubility laws as applied to tertiary phosphates. *Ibid.* 63: 677–706.

(31) Wrangell, M. von, and Mayer, L. 1926 V. A study of the root soluble portions of soil phosphoric acid, a comparison of the Neubauer method with the expressed solution colorimetric method. *Ibid.* 63: 739–775.

Brief abstracts of articles I-V appeared in *Chem. Abs.* (1927) 21: 2752, 2857, 4003.

THE AVAILABILITY OF THE NITROGEN IN FARM MANURE UNDER FIELD CONDITIONS

A. FLOYD HECK

University of Wisconsin[1]

Received for publication July 14, 1930

A great deal of work has been reported on the availability of the nitrogen from farm manure. By far the greater part of this work has been conducted in the laboratory or greenhouse. A recent paper (5) from this laboratory is of this nature, and shows that little other than the liquid manure or ammonia nitrogen is available for plant growth. These results support the findings of Barthel and Bengtsson (1, 2) who report that only the ammonia nitrogen in manure is available. Reliable field data on this subject are not so plentiful. Most of the field data with farm manure are reported in acre yields from a given application of manure in tons an acre and as a rule neither the amount of nitrogen recovered in the crop nor the amount and kind of nitrogen added in the manure are reported. The Ohio (7) results are somewhat of this nature but if average composition for the crop yields are used, the results show an availability of the nitrogen in the manure varying from nothing to about 30 per cent. Figures obtained in this way are too indefinite to be of any great value. The work done at the New Jersey (6) station furnishes more data and shows that during a period of 20 years, from 13 to 21 per cent of the nitrogen in cattle manure is recovered in the crops grown after its application. This work was done during four consecutive 5-year periods and is supplemented by chemical analyses. In this work lime shows a slight detrimental effect which increases with time, but the availability of the manure nitrogen used without lime increased slightly in the later periods of the experiment. In this experiment the amount of manure used was much in excess of the amount that would ordinarily be used in general farming.

From Rothamsted, Hall (3) reports a 30 per cent utilization of manure nitrogen by mangels, which is much higher than those from the New Jersey station. Hansen (4) reports work from Aarslev (Denmark) showing a recovery of from 33 to 58 per cent of the nitrogen applied in farm manure. This work was done with carefully analyzed manure which was applied at the rate of 1/2, 1, and 1 1/2 units (1 unit equals 9,000 kgm.) per hectare. Crops were then grown in rotation and the nitrogen recovery obtained by analyses of the crops produced.

These figures, taken together, show a variation in nitrogen recovery from nothing to as much as over half of the total nitrogen in the manure. This

[1] From the department of soils.

variation is no doubt due to the variation in the quality of the manure and also to the way it was handled in its application. From previous work (5) it is logical to conclude that the value of farm manure is in direct proportion to the amount of liquid manure nitrogen which it contains when it is incorporated into the soil, and as a general rule low availability of nitrogen from farm manure means either a heavy loss of the liquid manure or ammonia nitrogen or the incorporation of excessive amounts of straw as bedding.

FIELD EXPERIMENT WITH FARM MANURE FOR SPRING BARLEY

A field study on the availability of the nitrogen in farm manure was conducted with spring barley. Fermented and fresh cattle manure and artificial manure were used. The dung and liquid manure were used alone, together, and also combined with straw as bedding. The liquid and solid portions of the manure were each obtained separately, analyzed, and then used in the proportion (by weight) of 74 per cent dung, 20 per cent urine, and 6 per cent straw. The straw was mixed grain straw cut into 1/2-inch lengths and contained 1 per cent nitrogen. The fresh manure was applied to the land immediately and either plowed down at once or allowed to dry, as the case might be. In one case it was allowed to stand in a closed container for three days and ammonify before application. The ingredients for the fermented manure were mixed together in the fresh condition and then allowed to ferment in a closed container for six weeks before application. The two artificial manures were made 18 months previously, by allowing moist grain straw to decompose, one with and the other without the application of ammonium sulfate. This manure was well rotted and contained very little other than organic nitrogen.

The plots were laid out in the field on a light sandy soil, rather low in organic matter and nitrogen. The plots were 6 by 11 feet and the manure was applied to this area at the rate of 12 tons (wet) of complete manure to the acre. Seven feet (14 rows, 6 inches apart) on one end of each plot was seeded to barley and the other 4 feet was kept clean cultivated (fallow). Two rows on the ends and 12 inches on the sides of each plot were used as border so that the harvested plot was exactly 4 by 5 feet, 20 square feet, or 10 rows 6 inches apart and each 4 feet long. The plots were grouped in two series of 7 plots each, making a rectangular tract 22 by 42 feet with the two control plots on opposite corners. The barley was planted in a solid block 14 feet wide in the middle of this tract, leaving the outside end of each plot clean cultivated for nitrate studies. All operations were carefully done by hand. Figure 1 shows the plan of these plots.

The kinds of manure used and the methods of handling the manure for the various plots are as follows:

1. Control.
2. Complete fermented manure—plowed down at once.
3. Complete fermented manure—received 1/2 inch of rain at once, was then allowed to dry before plowing down.

4. Complete fermented manure—allowed to dry before plowing.
5. Complete fremented manure—top-dressed and disked in at once.
6. Artificial manure, made without ammonium sulfate, plowed down at once.
7. Artificial manure, made with ammonium sulfate, plowed down at once.
8. Fresh manure, urine only—plowed down at once.
9. Fresh manure, dung only—plowed down at once.
10. Fresh manure, dung and urine—plowed down at once.
11. Fresh manure, dung, urine, and straw—plowed down at once.
12. Fresh manure, dung, urine, and straw—allowed to dry before plowing down.
13. Fresh manure, dung, urine, and straw, ammonified three days before application—allowed to dry before plowing down.
14. Control.

All plots received superphosphate and potassium chloride. These applications were made in such a way that the phosphorus and potassium added in

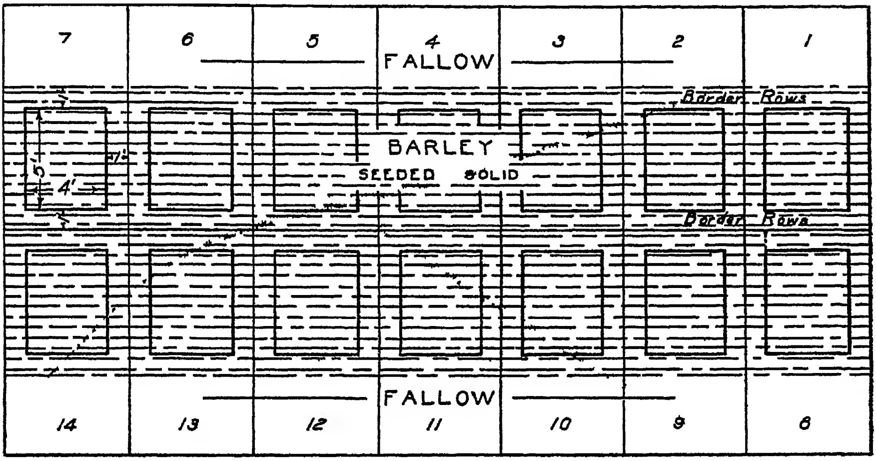


FIG. 1. PLAN OF SMALL FIELD PLOTS USED TO DETERMINE THE NITROGEN AVAILABILITY OF MANURE

the fertilizer, plus that added in the manure, would be approximately the same for all plots and this amount high enough to eliminte as much as possible the phosphorus and potassium factors. The manure was to have been applied at the rate of 12 tons an acre, but a slight error in measurement at the time, of its application caused this, as well as the P and K fertilizer, to vary somewhat. These have been figured to accurate acre applications and these figures, together with the amounts of nitrogen applied per acre in the manure, are given in table 1.

The manure for plots 3, 4, 12, and 13, on which the manure was allowed to dry before plowing, was applied during the afternoon of April 22. The manure was spread evenly over the plot and allowed to dry until April 26 when the manure was applied to the other plots and all were plowed. Plot 5 was top-dressed just before seeding.

The weather conditions were not favorable for drying and loss of nitrogen, as there was only about 15 hours of fair drying weather. This was followed by two days of rain (1.15 inches) accompanied by low temperature and high relative humidity. Table 2 shows the weather conditions during this period.

The barley was seeded with a Planet Junior garden drill in rows 6 inches apart running across the plots. After the barley had come up, any vacant spaces in

TABLE 1

Applications per acre of fertilizers and manure for barley

PLOT NUMBER	SUPER-PHOSPHATE	KCl	MANURE	NITROGEN		
				Urine nitrogen	Ammonia nitrogen	Total nitrogen
	pounds	*pounds*	*tons*	*pounds*	*pounds*	*pounds*
1	355.0	266.0	.....	....	....	.....
2	98.5	98.5	11.82	....	72.5	137.1
3	97.0	97.0	11.66	....	71.4	135.1
4	95.7	95.7	11.5	....	70.4	133.2
5	94.2	94.2	11.33	....	69.3	131.2
6	93.0	93.0	11.16	....	....	136.0
7	88.0	88.0	10.56	....	....	171.1
8	264.0	88.0	0.211	61.7	....	61.7
9	93.0	186.0	8.258	....	....	59.0
10	94.2	94.2	11.33	65.3	....	125.1
11	95.7	95.7	11.5	66.4	....	141.5
12	97.0	97.0	11.6	67.25	....	143.5
13	98.5	98.5	11.82	72.6	....	151.7
14	355.0	266.0	.....	....	....	.....

TABLE 2

Weather conditions during drying of manure

	MEAN TEMPERATURE	RAIN	WIND VELOCITY	RELATIVE HUMIDITY
	°F.	*inches*	*miles an hour*	*per cent*
April 22	50	0	3.8	47
April 23	52	0	6.9	47
April 24	48	0.41	7.8	88
April 25	48	0.74	14.4	83
April 26	54	0	9.6	53

the rows longer than 3 inches were planted with sprouted barley. This procedure insured a very uniform stand, as may be seen in table 6.

The fallow ends of the plots were used for nitrate study. Beginning May 15, samples for nitrates were taken at 15-day intervals up to June 29. The samples were taken in three parts, first 6 inches, second 6 inches, and second foot, and each sample was a composite from two borings. The two borings of each sample were mixed and sifted while moist through an 8-mesh screen. A

sample of about 100 gm. was rapidly dried on the hot plate or in the oven at a temperature not to exceed 60°C. Nitrate determinations were made by the

TABLE 3

Seasonal accumulation of nitrate nitrogen under fallow on the manure plots, and also the residual nitrate nitrogen under barley at harvest

Pounds per acre 2 feet

PLOT NUMBER	NITRATE NITROGEN UNDER FALLOW				RESIDUAL NITRATE NITROGEN UNDER BARLEY AT HARVEST JUNE 29
	May 15	May 30	June 14	June 29	
1	9.2	17.5	18.0	19.0	6.8
2	15.2	68.4	103.0	138.0	7.0
3	19.8	66.4	61.0	81.0	6.25
4	17.3	61.6	47.6	65.0	6.75
5	17.6	89.0	121.0	115.0	6.25
6	6.4	9.5	14.6	23.0	6.25
7	7.0	9.0	15.0	42.7	3.75
8	81.1	70.8	69.6	70.5	....
9	8.1	11.9	12.1	21.5	....
10	32.8	41.6	28.9	149.0	....
11	17.4	51.6	52.5	79.0	....
12	24.9	29.4	37.6	60.6	....
13	22.7	20.9	42.5	67.6	....
14	9.5	13.0	18.3	34.4	....

TABLE 4

Yields, nitrogen contents, and amounts of nitrogen per acre in the barley crop

PLOT NUMBER	TOPS			ROOTS			STUBBLE		
	Weight per acre	Nitrogen content	Nitrogen per acre	Weight per acre	Nitrogen content	Nitrogen per acre	Weight per acre	Nitrogen content	N'
	pounds	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per*	
1	1,118	1.35	15.1	504	1.29	6.5	206		
2	4,461	1.54	68.7	682	1.32	9.0	3r		
3	3,660	1.66	60.8	787	1.46	11.5			
4	3,615	1.48	53.5	687	1.35	9			
5	4,351	1.76	76.6	672	1.57				
6	1,101	1.60	17.6	345					
7	1,124	1.56	17.5	360					
8	3,418	1.45	49.5						
9	822	1.48	12.2						
10	3,926	1.81	7						
11	3,642	1.66							
12	3,082	1.71							
13	2,479	1.49							
14	943	1.61							

phenol-di-sulfonic acid m
the flocculating agents.

acre 2 feet, based on 4,000,000 pounds per acre foot. The determinations seldom showed more than 6 pounds of nitrate nitrogen in the second foot,

TABLE 5

Nitrogen applied in manure and that recovered from it by the barley crop

PLOT NUMBER	NITROGEN APPLIED IN MANURE			TOTAL NITROGEN IN BARLEY CROP	NITROGEN RECOVERED FROM MANURES	
	Urine nitrogen	Ammonia nitrogen	Total nitrogen		Of urine or NH_3-N	Of total nitrogen
	pounds per acre	*pounds per acre*	*pounds per acre*	*pounds per acre*	*per cent*	*per cent*
1	.....	....	.....	23.4	....	....
2	.....	72.5	137.1	81.0	80.2	42.4
3	.....	71.4	135.1	76.0	74.6	39.4
4	.....	70.4	133.2	66.1	61.5	32.5
5	.....	69.3	131.2	91.0	98.5	52.0
6	.....	....	136.0	23.6	....	0.6
7	.....	....	171.1	24.2	....	0.8
8	61.75	....	61.75	65.2	68.7	68.7
9	.....	....	59.0	20.2	....	....
10	65.3	....	125.1	91.2	104.7	54.7
11	66.4	....	141.5	76.0	80.2	37.6
12	67.25	....	143.5	69 2	69.0	32.3
13	72.6	....	151.7	50.6	38.0	17.7
14	.....	....	.....	22.2	....	....

TABLE 6

The effect of manure on the tillering of barley

PLOT NUMBER	PLANTS PER SQUARE FOOT	TILLERS	
		Per square foot	Per plant
1	16	24.0	1.5
[illegible]	13.5	44.5	3.3
[illegible]	16.5	48.0	2.9
[illegible]	15.5	40.5	2.6
[illegible]	16.5	60.0	3.6
[illegible]	13.5	19.0	1.4
[illegible]	[illegible]	21.5	1.4
[illegible]	[illegible]	42.5	3.4
[illegible]	[illegible]	17.0	1.4
[illegible]	[illegible]	52.0	3.0
[illegible]	[illegible]	43.0	3.0
[illegible]	[illegible]	[illegible]	2.7
[illegible]	[illegible]	[illegible]	2.5
[illegible]	[illegible]	[illegible]	1.3

nitrate during the experi-
here the urine alone was
s found in the second foot

at the first sampling, which seemed to indicate leaching of the nitrogen in the urea stage. Table 3 gives the nitrate nitrogen in pounds per acre, 2 feet, at the various sampling dates.

The barley was harvested on June 29. At this time it was fully headed. The crop from the entire plot (20 sq. ft.) was dried, weighed, ground, and sampled for nitrogen determination. The soil from two rows of barley each 2 feet long (2 q. ft). and 8 inches deep, was carefully removed from each plot and the roots and stubble washed out. When the roots and stubble were partially dry all foreign matter was carefully removed and the fibrous roots separated from the stubble. Both were dried, weighed and ground for analysis. The amounts of nitrogen were determined in the tops, roots, and stubble, and the proportions of the manure nitrogen recovered by the barley calculated. The number of plants to the square foot and the tillers to each plant for each plot were determined. These data are given in tables 4, 5, and 6.

DISCUSSION

The recovery of nitrogen from the manure in this work varied from nothing in case of the dung and artificial manures to over half the total or all of the ammonia or urine nitrogen in cases of the top-dressed fermented complete manure and the fresh urine-dung mixture. There are several factors which seem to influence the availability of manure nitrogen. Among these may be mentioned; the ratio of ammonia or urea nitrogen to the total, the proportion of bedding or energy material in the mixture, and the methods of handling. The last factor would include storage, whether fresh, ammonified, or completely fermented and also methods of application, whether top-dressed or plowed under, or whether allowed to dry or worked in immediately after spreading.

Table 5 shows that where the complete manure is top-dressed and disked in at once and where the dung and urine are plowed down at once, all of the ammonia or urine-nitrogen is recovered in the barley. Both complete fresh manure and complete fermented manure when plowed under at once give a recovery of 80 per cent of the soluble nitrogen. With the fermented manure, plowing down reduces the recovery 18 per cent as against top-dressing. Two factors may have operated here to give these results. The shallower incorporation places and holds the soluble nitrogen in the zone of the young plant roots so that the plant is able to obtain its nitrogen earlier. The top-dressed plot showed this to a marked degree from the earliest growth period. In the second place, the shallower incorporation is more conducive to nitrification earlier in the season because of the higher temperature and better aeration. This is shown by the nitrate curves in figure 2. The nitrate curve for the top-dressed manure is from 2 to 5 days ahead of the curve for the plot where the manure is plowed down at once, and this condition was reflected in the crop from the early seedling stage. The addition of fresh straw gives even a greater depression than plowing down the fermented manure. This depression is caused by the growth of soil microorganisms as a result of the increased amount

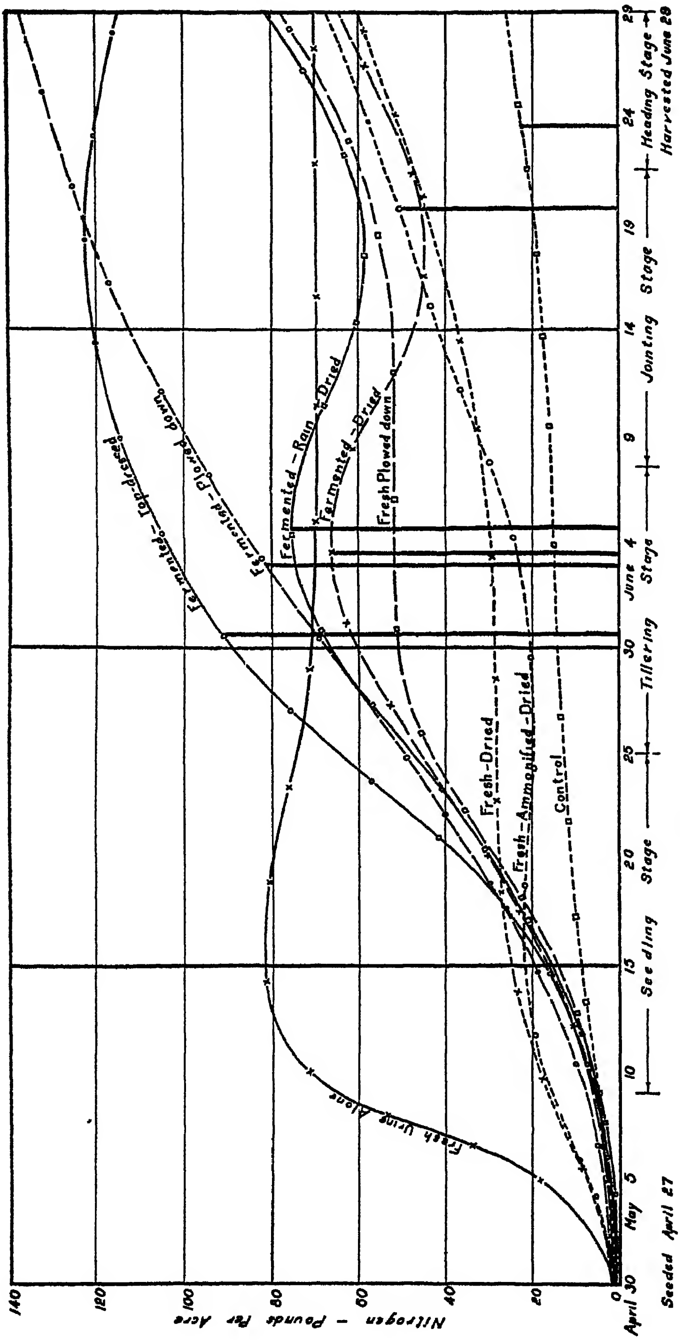


FIG. 2. NITRIFICATION CURVES ON FALLOW DURING THE GROWTH PERIOD OF SPRING BARLEY ON SIMILARLY MANURED LAND

(The heavy vertical lines represent the earliest date during the growing period when the amount of nitrate nitrogen in the fallow equals the total nitrogen in the crop at harvest time.)

of readily available energy material. This biological activity brings about a slowing up of nitrate accumulation during the tillering and early jointing stages.

The depression in nitrogen recovery, caused by the drying of the manure after spreading in the field, is probably due to the volatilization of ammonia nitrogen, and the amount of the depression depends upon the previous handling of the manure and the amount of drying to which it is exposed. In case of the fermented manure, $1\frac{1}{2}$ days' drying depresses the recovery 19 per cent, but when this manure is spread immediately before a $\frac{1}{2}$-inch rain, the depression is only about one-third of this amount. The depression from the drying of fresh manure is only about one-half that of the fermented manure. In the case of the fresh manure, the nitrogen is in the form of urea with less chance of loss by volatilization. The depression in case of the fresh manure is no doubt due to the loss of ammonia formed during the drying process. Where the fresh manure is allowed to stand for three days and ammonify and is then subjected to drying for $1\frac{1}{2}$ days after being spread, only about 38 per cent of the nitrogen in the liquid manure is recovered in the crop. The greater loss in case of the freshly ammonified manure in comparison with the fermented manure is accounted for by the fact that the nitrogen in the former is largely in the form of ammonium carbonate, whereas in the latter case, part of the nitrogen is held as the ammonium salts of volatile organic acids such as acetic and butyric. As the salts of these acids, the ammonia is less volatile than as the carbonate and hence the loss is slower in the fermented than in the freshly ammonified manure.

There is practically no recovery of the nitrogen in the artificial manures, although they contained as much nitrogen or more than the animal manures and were in a well-rotted condition. The fresh dung, even when used alone, gives a depression below the controls. Practically all of the nitrogen in these manures is in the organic form and they also contain enough carbonaceous energy material to depress the recovery of any available nitrogen that may be present. This is particulally true of the dung. It is rather strange to note that the nitrogen from the liquid manure, when used alone, is recovered only to the extent of 68 per cent, but when used in conjunction with dung, which gives a depression, the recovery is raised to 104 per cent of the liquid manure nitrogen. This apparent discrepancy may be explained by leaching. Nitrate determinations on the liquid manure plot indicate that there is considerable leaching of the urea nitrogen apparently before it has a chance to change to ammonia. In this particular case at a very early date a large quantity of nitrate nitrogen was found in the second foot. When the liquid manure is used in conjunction with dung it is held more or less mechanically in the surface soil so that the plant is able to utilize more of the nitrogen at an early date.

Nitrate relations. A study of the nitrate curves in figure 2 in relation to crop growth and the availability of manure nitrogen is very interesting. The four plots on which fermented manure was used fall into two pairs, according to the nitrate curves, the two which were allowed to dry after spreading and

the two worked into the soil at once. Of the two worked in at once, the top-dressed plot is the higher in the seedling and tillering stages. Plowing down slows up the nitrification rate during these stages of growth and causes a

TABLE 7

Rainfall for April, May, and June, 1929, at Madison, Wisconsin

DAY	APRIL	MAY	JUNE
	inches	*inches*	*inches*
1	0.31	T	0
2	T	0	0
3	0	0	0
4	0.01	0	0
5	0.02	0	T
6	1.09	0	0
7	T	0	T
8	0	0.02	0
9	0	0	0
10	0.27	0.44	0
11	0.45	0.17	0.34
12	T	0	T
13	0	0	0.36
14	0	0.11	0.03
15	0	0.05	0
16	T	0	1.97
17	T	0.06	T
18	0	T	0.98
19	0.20	0	0.05
20	0.03	0	0
21	0	0	0
22	0	T	T
23	0	0.35	T
24	0.41	0	0.05
25	0.74	0	0.07
26	0	0.03	0.04
27	0.53	0.05	0.45
28	0.02	0	0
29	0.10	0.03	0
30	0.11	0	0.01
31		0	

Trace.

lesser amount to be recovered by the barley, although the nitrate nitrogen on this plot is higher at a later date. The nitrates for the two plots where the manure is dried are again parallel, with the one which received rain always

higher and at the same time giving the greater recovery. This is because of the lesser loss of nitrogen on drying after the rain. The shape of these curves is significant. The rise during the seedling and tillering stages is slower than where the manure is worked in without drying. The drop coming during the late tillering and early jointing stages may be caused by the growth of higher soil fungi which seem to show up at about this time. During this period these organisms are using the energy material of a cellulose nature in the manure and at the same time using the nitrogen more rapidly than nitrification is going on. Later in the season the nitrate curves on these two plots take an upward trend after the rains of June 16 and 18. Better conditions for nitrification, due to moisture, coming perhaps on the downward growth curve of the higher soil fungi, may be responsible for this upward trend, but coming so late in the life of the plant, it is of little benefit. The effect of the growth of the higher soil fungi may be seen in the top-dressed plot. During the last two weeks, fruiting bodies of *Stropharia semiglobota* and a species of *Nectria* apleared in abundance and at the same time there was a downward trend in the nitrate curve.

In each of these four plots the earliest point in the nitrate curve where the amount of nitrate nitrogen corresponds to the amount of nitrogen recovered by the barley, falls some place during the tillering period. It seems also that the earlier this comes, the greater the amount of nitrogen is recovered in the crop. On the control and plot 13 (ammonified and dried) the nitrate curves are low during the seedling and tillering stages and this point comes late in the jointing stage with a low recovery of the nitrogen applied. On the other hand, fresh urine alone gives a high nitrate curve early in the seedling stage of the plant, with a low percentage recovery, partially due perhaps to leaching of urea-nitrogen and partially to the bulk of the nitrates coming too early in the period of plant growth. From these data it appears that there is a relation between the shape of the nitrate curve and the growth of plants and their utilization of nitrogen. If the rise comes too early or before the late seedling and tillering stage, there is a tendency for leaching losses to take place. If, on the other hand, the nitrate curve rises slowly and is low during the seedling and tillering stages, the plant passes its stage of most active nitrogen assimilation and the result is a low yield and a low nitrogen assimilation. A nitrate curve similar to that of plot 5 seems to be the one most conducive to maximum nitrogen assimilation and to maximum plant growth, and this curve perhaps approached more nearly the growth curve of the plant than any of the others. It would follow then that the nitrate curve which most nearly conforms to the growth curve of the plant during its early stages, both in regard to time and amount of nitrate accumulation, is the one which is the most likely to produce the maximum plant growth. From a practical standpoint the curves in figure 2 show that manure should be plowed under a week or two earlier than it is top-dressed and for the best results the liquid manure should be applied after the crop is up.

The kind and amount of the material used as bedding are important fac-

tors in the availability of manure nitrogen. Table 5 shows that the addition of 6 per cent by weight of straw to the liquid and solid manure mixture reduces the availability of the liquid manure nitrogen over 20 per cent. Wood shavings used as an absorbent are even more detrimental than straw. The depressing effect of these substances is due to the use of ammonia and nitrate nitrogen by soil fungi, which at the same time use certain carbonaceous materials in the straw or shavings as energy for their growth. Niemeyer (8) has shown that the use of peat as an absorbent has two distinct advantages over straw or shavings: First, it is a better absorbent and more of the liquid manure is saved and applied to the field, and, second, there is very little energy material in the peat for the growth of soil fungi and as a result the depressing effect from this source is largely eliminated.

It is very possible that the percentage recoveries reported in this paper will hold true only for manures of a similar ratio of soluble to insoluble nitrogen. It is also very likely that a given amount of energy material of a cellulose nature will use a given amount of soluble nitrogen and not a percentage of the amount present. In cases of low water-soluble or ammonia nitrogen it is just possible that all of it may be used by the soil microörganisms and none recovered by the plant.

SUMMARY

A field study of the availability of the nitrogen in cattle manure was made with spring barley and the following points seem to be outstanding:

The amount of nitrogen recovered in the barley crop was never greater than the amount of ammonia or liquid manure nitrogen applied. The results indicate that only the ammonia or liquid manure nitrogen is available for the first crop after the manure is applied to the soil. Little if any of the water-insoluble nitrogen in the manure is recovered in the first crop.

When liquid manure is applied alone before the crop is seeded, the recovery is less than when applied in conjunction with the dung. This difference seems to be due in part to leaching of the urea before its change to ammonia. If liquid manure is to be used alone it is perhaps best applied to the growing crop and worked into the soil at once.

Complete manure turned under immediately on spreading gives a recovery of 80 per cent of the urea or ammonia nitrogen which it contains. There is no difference in the availability of nitrogen between the manure fermented in storage and the fresh manure when they are turned under immediately on spreading.

Top-dressing gives a higher availability than plowing down. The nitrification of manure top-dressed and disked in is more rapid than when it is plowed under.

Measured in terms of non-recovery, the greatest loss of nitrogen in spreading manure and allowing it to dry or partially dry before being plowed under, occurs with manure which has ammonified but not fermented. The next greatest loss is from the completely fermented manure. The fresh manure gives a still smaller loss under these conditions, and where the fermented manure is spread just previous to a rain the loss from drying is not more than a few per cent. The better the conditions for drying and the greater the proportion of the nitrogen in the form of ammonium carbonate, the greater the loss of nitrogen in handling, spreading, and subsequent drying.

The addition of straw to manure decreases the amount of nitrogen recovered in the first crop. Six per cent of straw used as bedding in the manure reduced the recovery more than 20 per cent.

Conditions which give a nitrification curve most similar to the growth curve of the plant and furnish sufficient nitrogen for rapid growth during the seedling and tillering stages, are most conducive to high yields and the greatest recovery of manure nitrogen.

REFERENCES

(1) Barthel, C., and Bengtsson, N. 1924 Bidrag till frågan om stallgödselkvävets nitrifikation i åkerjorden. IV. Meddel. Centralanst. Försöksv. Jordbruksområdet. (Sweden) 269.

(2) Barthel, C., and Bengtsson, N. 1926 Bidrag till frågan om stallgödselkvävets nitrifikation i åkerjorden. V. Meddel. Centralanst. Försöksv. Jordbruksområdet. (Sweden) 311.

(3) Hall, A. D. 1917 The Book of the Rothamsted Experiments. New York.

(4) Hansen, N. A. 1928 Gødningsforsøg paa Forsøgsstationen ved Aarslev 1911–1926. *Tidsskr. Planteavl.* 34: 373–523.

(5) Heck, A. F. 1931 Conservation and availability of the nitrogen in farm manure. *Soil Sci.* 31: 335–363.

(6) Lipman, J. G., A. W., and Prince, A. L. 1928 Field experiments on the availability of nitrogenous fertilizers, 1923–1927. *Soil Sci.* 26: 1–25.

(7) Maintenance of soil fertility, The 1924 Ohio Agr. Exp. Sta. Bul. 381.

(8) Niemeyer, P. 1929 Zersetzung und Wirkung von Stroh- und Torfstreustalldünger im Boden. *Landw. Vers. Sta.* 108: 305–348.

PLATE 1

Barley Growth as Affected by Various Fertilizer Treatments

Fig. 1. Barley on the control, no manure or nitrogen added.

Fig. 2. Barley from 12 tons an acre of artificial manure made from straw by the use of ammonium sulfate, and plowed down immediately after being spread.

Fig. 3. Barley from 12 tons an acre of complete fermented manure, plowed down immediately after being spread.

Fig. 4. Barley from 12 tons an acre of complete fermented manure,—allowed to dry in the field before being plowed down

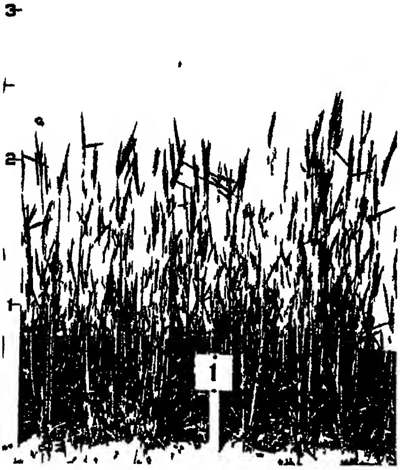


Fig. 1

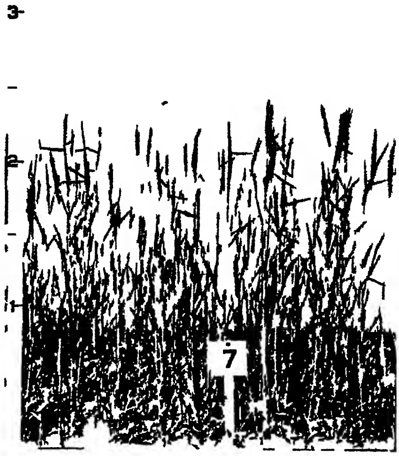


Fig. 2

Fig. 3

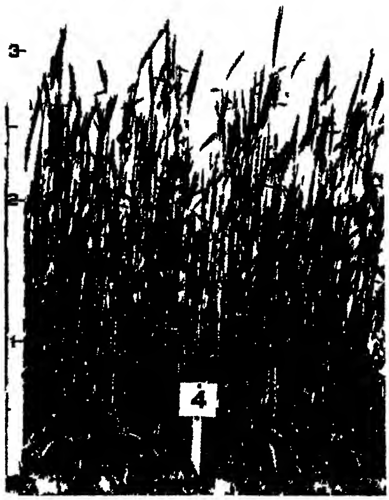


Fig. 4

DETERMINATION OF SOIL ORGANIC MATTER

C. J. SCHOLLENBERGER

Ohio Agricultural Experiment Station

Received for publication December 29, 1930

Several years ago, the writer described a simple procedure for the approximate determination of soil organic matter (5), based upon the oxidation of the sample by heating with concentrated sulfuric acid containing excess chromic acid at such a rate that the temperature reaches 175° C. in 90 seconds, cooling at once, diluting and titrating the chromic acid not consumed in oxidizing organic matter by a standard solution of ferrous ammonium sulfate with diphenylamine as internal indicator. Although no great degree of precision was claimed for this method, it seems to have found considerable favor, judging from the number of investigators who have reported results obtained by its use. Degtjareff (1) reports a study of the method, substantially as originally described except that more care was taken to have the time and temperature uniform for all tests, by heating in a sulfuric acid bath at 165° C. for 10 minutes. He also advises close attention to the color change at the endpoint. His results as reported show that the method is capable of considerable accuracy, and for many purposes the indications obtained in this way should be quite as satisfactory as those by procedures for which great precision is claimed. In the same paper, an alternate procedure is described which differs principally from the original method by the inclusion of hydrogen peroxide in the oxidizing mixture and the omission of heating. As in the first method, the excess of oxidizing agent is titrated with standard ferrous ammonium sulfate and diphenylamine is employed as internal indicator.

A paper presenting the results of a very complete study of the indicator properties of diphenylamine by Kolthoff and Sarver (3) has appeared recently. The data obtained and conclusions drawn from a study of the same indicator in ferrous iron-dichromate titrations have been published (6). The author's experience has been in agreement with the conclusions of Kolthoff and Sarver that diphenylamine, or rather its oxidation product, is a true oxidation-reduction indicator developing a characteristic blue color at a potential of about 0.51 volt as measured by a standard set-up for electrometric titrations including a saturated potassium chloride-calomel electrode, at about 25°C. In the usual titration of a ferrous salt by dichromate, the oxidation-reduction potential is buffered by the mixture of ferrous and ferric ions and the potential mentioned is reached at a comparatively flat portion of the titration curve; the diphenylamine color therefore appears prior to complete oxidation of iron and

increases in intensity as the titration is completed. On back-titration with titanous chloride, the color fades gradually until the potential has fallen to that point, when it disappears entirely. In back-titration of excess chromic acid with ferrous salt solution, however, the potential drop after complete reduction of chromic acid is very gradual, since Fe'' cannot reduce Fe''', and a considerable excess of ferrous salt is necessary to take the potential below the point at which the indicator still shows color. Under these circumstances, the endpoint is not sharp, being indicated by a pronounced fading rather than a total disappearance of blue, as was noted when the procedure was first described (5).

The author has found that by the addition of either phosphoric or hydrofluoric acid, Fe''' is inactivated by formation of slightly dissociated salts, the buffer effect greatly diminished, and the potential consequently lowered. At the point of complete oxidation of iron or reduction of excess chromic acid the titration curve is asymptotic, and an extremely small increment of the titrating solution is sufficient to take the oxidation-reduction potential past the critical value for color change. The endpoint is then very sharp and easily seen, since another effect of the inactivation of Fe''' is the bleaching of the normal yellow color. Knop (2) states that the presence of sufficient phosphoric acid to destroy the yellow color of ferric salt is advantageous, but gave no other explanation for its action. The author did not use it, as results seemed sufficiently exact without it. Also, it has been his custom to determine available phosphoric acid in soils by the very sensitive ceruleomolybdate method at the same time that the test for organic matter was made, and the use of phosphoric acid in one determination is undesirable since it may lead to error from contamination of the other. Hydrofluoric acid is of course without this objection, but is unpleasant and even dangerous to handle. Szebellédy (7) states that the diphenylamine endpoint is more satisfactory in the presence of ammonium fluoride, but there seems to be no reason why sodium fluoride should not serve as well. Lang and Kurtz (4) found sodium fluoride a desirable addition in a somewhat similar titration, but state the use of potassium fluoride was not satisfactory as it seemed to interfere with color development. The author has tried only hydrofluoric acid and sodium fluoride, and prefers the latter. All fluorides are of course destructive to glassware, but the error introduced by solution of Pyrex glass during the brief time necessary for titration is of no practical consequence, being very small and compensated by the blank determination.

Besides the lack of sharpness in the diphenylamine endpoint, which can be overcome by addition of phosphoric or hydrofluoric acid, the indicator has several other peculiarities which have caused some to condemn it after brief experience. The most disconcerting of these is the occasional failure to develop the blue color when added to a solution containing excess chromic acid; in such cases it is always noticed that on addition of the first drop of ferrous sulfate the color appears, and increases as the next two or three drops are added. The explanation for this puzzling behavior is that not diphenylamine itself, but a product of its partial oxidation, is the indicator, as Kolthoff and Sarver

(3) have pointed out. Like many other organic substances, diphenylamine is not very readily oxidized by chromic acid alone in cold dilute acid solution, but if a trace of substance readily oxidized is added, "induced oxidation" of the more resistant substance takes place. Other instances of the same phenomenon and theories explaining it are given by Lang and Kurtz (4). Another objectionable feature is the appearance of a green oxidation product instead of the blue indicator substance. This is especially likely to happen when diphenylamine is added to a solution containing excess chromic acid. In these respects also, the trouble is much reduced by addition of phosphoric or hydrofluoric acid, and by limiting the amount of diphenylamine added to the solution. Two or three drops of a one-half per cent solution is ample for the production of an intense endpoint in a 200-cc. volume, and more is undesirable.

Extended experience in the determination of organic matter by the chromic acid oxidation method under discussion has indicated that it is not at all necessary to prepare the solution of dichromate in concentrated sulfuric acid as originally specified, although it is desirable to do this if many determinations are to be made at the same time. Very good results are obtained by weighing out the proper amount of pure potassium dichromate, previously pulverized and dried, transferring it to the test tube, adding the sample, and finally washing down the walls of the tube with 10 cc. pure sulfuric acid, and heating at once. This is a useful expedient in the examination of samples high in organic matter, as the usual 0.5-gm. sample may be used with sufficient oxidizing agent and only the usual amount of acid. By this procedure also, one is able to determine both the blank and titer of a partially deteriorated ferrous ammonium sulfate solution, still of sufficient strength to be usable. In this case, potassium dichromate is the primary standard; it is well adapted to the purpose, as it is easily obtained in pure form, is anhydrous and non-hygroscopic. The equivalent of 1 cc. 0.2 *N* solution is 0.009807 gm., equal to 0.0006 gm. carbon or approximately 0.0012 gm. organic matter. For the determination of organic matter in a 0.5-gm. sample of average soil, the equivalent of 20 cc. 0.2 *N* solution, 0.1961 gm. $K_2Cr_2O_7$ may be used. The weight of the salt taken may be varied as required, within reasonable limits.

It is recommended, therefore, that the procedure for determination of organic matter in soil originally described be amended as follows: Proceed as originally directed, or according to the modification suggested in the last paragraph, or one of the procedures described by Degtjareff (1), up to the point at which the solution is transferred to a beaker for titration. Dilute with recently boiled and cooled water, to at least 10 but not more than 15 times the volume of concentrated sulfuric acid present, and cool. To this solution add, in volume equal to half that of concentrated sulfuric acid in the solution, either 85 per cent phosphoric acid or 48 per cent hydrofluoric acid or the approximate equivalent of the latter in powdered sodium fluoride, i.e., 5 gm. NaF for 10 cc. concentrated sulfuric acid. Add two or three drops of diphenylamine indicator solution and titrate with standard ferrous ammonium sulfate solution to total disappear-

ance of blue from the green solution. Deduct the burette reading from that of a blank titration conducted under precisely similar conditions except that no sample is used, and from the number of cubic centimeters of iron solution equivalent to organic matter in the sample calculate the amount present. One cubic centimeter 0.2 N ferrous ammonium sulfate solution is equivalent to approximately 0.25 per cent organic matter in a 0.5-gm. sample, or 2.5 tons in an acre.

REFERENCES

(1) Degtjareff, W. Th. 1930 Determining soil organic matter by means of hydrogen peroxide and chromic acid. *Soil Sci.* 29: 239–245.

(2) Knop, J. 1924. Diphenylamine as indicator in the titration of iron with dichromate solution. *Jour. Amer. Chem. Soc.* 46: 263–269.

(3) Kolthoff, I. M., and Sarver, L. A. 1930. Properties of diphenylamine and diphenylbenzidine as oxidation-reduction indicators. *Jour. Amer. Chem. Soc.* 52: 4179–4191.

(4) Lang, R., and Kurtz, F. 1929. Maszanalytische Bestimmung von Mangan als Manganisalz nach einem neuen Verfahren. *Ztschr. Anorgan. Allg. Chem.* 181: 111–120.

(5) Schollenberger, C. J. 1927 A rapid approximate method for determining soil organic matter. *Soil Sci.* 24: 65–68.

(6) Schollenberger, C. J. 1931 Diphenylamine as indicator in the determination of iron in silicates. *Jour. Amer. Chem. Soc.* 53: 88–98.

(7) Szebellédy, L. 1930 Über die Bestimmung des Eisens mit Kaliumdichromat. *Ztschr. Anal. Chem.* 81: 97–103.

THE MODELING OF SOILS, AN AID IN THEIR STUDY

R. H. CARR[1]

Purdue University

Received for publication September 15, 1930

Many students of soils who have had extensive training in some of the more advanced lines, such as classification, hydrogen-ion determination, base exchange and colloidal characteristics, have been found very deficient in the ability to rate soils in the fields, either for their own benefit or for that of farmers, appraisers, or loan companies. This is believed to be partly because they have forgotten most of what they learned in the days when they made mud pies, dogs, and fences out of "dirt" and also because in later years they have been afraid of getting their hands dirty with mud or probably they have thought it useless to attempt to study a soil by its "feel."

Soil, from a technical standpoint, is a complex combination of compounds and it is difficult to rate many soils properly even with the help of refined instruments. There are certain characteristics of an elementary nature, however, the knowledge of which can be readily acquired by practice, although comparatively few people have succeeded in mastering field judging.

The writer was agreeably surprised some time ago at the thoroughness with which a certain farmer who had had little "book learning" had developed by practice when plowing and while the team was resting, the ability to detect differences in the texture, structure, color, and plasticity of the soils in various places and also to tell their influence on the tilth, water-holding capacity, and fertility of a certain rented farm. He had platted the locations of the changes in surface and subsoil conditions over the 110 acres. He would estimate the organic matter and test the "feel" to find sand particles by their grittyness and count the stones in each handful of soil. He estimated the silt by its floury feel, when dry and also noted that clayey material is rough and harsh when dry, and sticky and capable of being modeled when it is wet. He took much interest in making such molds as his fancy might suggest. The interest and enthusiasm in field studies of this tiller of the soil have been somewhat contagious and the writer has had some of the students interested in soils trying their skill in rating known and unknown soils. They, too have caught the spirit and have developed enough interest to make some very creditable molds. Several of them are shown in plate 1. The students soon found that some soils are too sandy or too grainy to stick together well, or if they stick together at first they will fall apart when dry. Some that did stay together were often too fri-

[1] Professor of Agricultural Chemistry.

able to outline any distinctive facial features. Plate I shows that in the center model, the soil particles are large enough to be apparent to the eye, whereas in the largest one the soil grains are very fine. The mold is smooth and hard now, but was very plastic when wet. In the smallest model there are fairly equal amounts of sand and clay; it was made from a well-recognized loam soil which neither molds well nor has a smooth finish.

There is a growing need and desire on the part of those interested in land to learn the field characteristics of soils and their meaning in terms of "good," "medium," and "poor," so that it may be possible to predict how the soil will stand a drought such as was experienced last year or how well it will drain off the excess water which was so serious a question two years ago. If the farmer has this information he will be able to prepare early for his spring crops.

Mechanical analyses of soils are necessary for the more detailed study of soil properties, but they are tedious to carry out (except by the hydrometer method). Hence, they are not much help to the man who does the plowing and who is financially concerned with drought and excess rainfall on the piece of land he has rented for the year. It has been found possible in a few cases by practice with a quart or more of soil from each of about two dozen samples, to develop considerable skill in detecting shades of differences in sand, silt, and clay. This requires no more practice than is needed to pitch a good game of horse shoes.

PLATE 1

MODELS MADE FROM SOILS

Left—from Miami surface soil; center—from Bainbridge subsoil; right—from surface soil of Clermont type. Photo by Dr. E. J. Kohl.

AUTHOR INDEX

SUBJECT INDEX